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# MORPHOGENESIS OF THE SHOULDER ARCHITECTURE
## PART I. GENERAL CONSIDERATIONS

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### INTRODUCTION

THE problem of limb development has received, during the past hundred years, the critical attention of a host of able morphologists, but as the sum of knowledge increases new light can be shed on the problem from time to time. One of the chief difficulties encountered in any such morphological investigation lies in the fact that even the lowest of our living vertebrates are probably as old phylogenetically as our highest and have had time to evolve many complicated and often misleading details of a tangential character, which may prove exceedingly difficult to evaluate.

In any study of the appendages equal regard must be accorded neurological, myological and skeletal features. No one of these can be discussed without a consideration of the others, from all possible angles that may apply, paying due regard to the alterations which these systems probably can or can not experience. Consideration must also be given to the evolution of posture, of locomotion, and to changes in environment. This the author attempts to do for the extrinsic and intrinsic structures of the pectoral girdle. He, of course, entertains no delusions that he can settle all of the points at issue, for many of them will remain controversial forever.

The basic facts concerning the pectoral appendage can be understood only by a scrutiny of all facts that may have a bearing on the subject, from the lowest to the highest forms. Hence the present report will be divided into a preliminary or general section, another on fish, and subsequent chapters on the higher vertebrates.

### EMBRYOLOGY

No proper discussion of nerve, muscle and bone can be undertaken without a brief exposition of their most salient embryonic features.

The neurological details most pertinent to the present study are phylogenetic rather than ontogenetic, and this system needs scant attention from the embryonic viewpoint. The nervous system is entirely derived from the neural plate of the ectoderm. That originally it was seg-

mental in character has frequently been claimed, but the more conservative school of thought holds that such suggestion of neuromerism as occurs is secondarily effected to conform to the metamerism of the somites, and recent experiments tend to uphold this viewpoint. The important feature is that invariably there is a pair of motor and a pair of sensory nerves given off by the spinal cord to each segment and that the motor root invariably innervates the myomere of the same segment. It was long considered that the early differentiation of the muscular system was actually influenced by the nervous system, but the myotome is laid down before the nerve reaches it. In fact the actual connection between nerve and muscle, by the motor end plates, is not effected until a stage at which practically all the muscle divisions have become differentiated (Cajal, 1928), and muscles would doubtless continue to develop to a postnatal stage even if their nerves could be completely extirpated.

The reason why a nerve filament always reaches the particular muscle for which it is destined is a matter that has received much controversial attention. It may be no more remarkable than the astonishing way in which some of the peripheral structures are laid down in an unvarying pattern, but the situation is different, for nerves, unlike most other anatomical details, actually grow by pushing out from a central area. They follow prescribed and often circuitous pathways with but relatively slight deviation from the normal. The subject is too much involved to discuss or even summarize here, but it is the opinion of Cajal, one of the greatest authorities on the question, that there is a definite neurotropic influence concerned: that specific stimuli for the orientation of the nervous cones of growth are particularly numerous in the embryonic period, the most active then appearing to be elaborated by the myotome, connective tissue, and neurones that are forming dendrites.

The fact that interests us here is that the proper nerve practically invariably reaches its proper muscle, but if this be prevented experimentally, then apparently there will be an effort made for innervation of the muscle by other nerves. It is not improbable that the latter can occur on rare occasions as an abnormality, and an appearance of heterotopic innervation be given.

Bone is classified as of two sorts, enchondral and intermembranous, but the basic difference may not be as great as is often implied, and a few osseous elements (sphenoid and temporal) are derived from both. Intermembranous bone was apparently first formed from mesenchyme at the base of such dermal elements as placoid scales and became extensive where originally there was need for surface strengthening, through the secretion of ossein and lime salts.

Enchondral bone can be divided into two categories, comprising structures derived essentially from mesoderm, and those with an origin largely ectodermal. The first of these groups may again be subdivided for convenience into those skeletal elements contributing to the chondrocranium, which, whatever may have been their original purpose, serve for the protection of vital parts, and those which have formed as the result usually of muscular stress. In the latter case the situation is almost invariably in intermuscular septa (exception, sesamoid bones), and the development is by condensation of (theoretically) any part of the mesenchyme. The second enchondral group, comprising the branchial cartilages, including Meckel's cartilage, palato-quadrate, etc., was found by Landacre (1921) to be derived, at least in urodeles, from ectoderm by differentiation of neural crest elements, and Stone (1926)

has recorded the formation of cartilage in transplanted neural crest. The exact significance of this is not yet understood.

Cartilaginous bone is formed in the lower vertebrates mostly by perichondral ossification invading cartilage, while in the higher forms enchondral ossification is the rule. In the cartilaginous fishes local calcification of cartilage frequently occurs.

All muscular tissue, except the intrinsic muscles of the eye ball and of the smooth skin musculature (of the skin glands), is usually conceded to be derived from embryonic mesoderm, although there appears to be some question concerning the precise derivation of the branchial components. In regard to the somatic musculature at least there is first formed dorsally, along both sides of the notochord, a pair of myotomes to each somite. These are sac-like, enclosing a myocoelic cavity which soon disappears. From them are derived all those back muscles originally situated dorsal to the lateral line organs, as well as cutis and osseous elements.

A frequent misconception regarding the development of the musculature is to the effect that the muscles ventral to the lateral line are formed by actual growth in that direction of the original, dorsally-situated myotomes. Conditions vary in different parts of the body, but in the anterior trunk at least there appears to be a lateroventral muscle mass entirely distinct from the dorsal myotome. Between the two there is a connective tissue septum, and tending further to separate them at early stages of phylogeny are the pronephros and its duct, and the lateral line structures. The lateroventral musculature differentiates by condensations of mesoderm progressively in a ventral direction, forming a lateral somatopleure, giving rise to the somatic musculature, and a medial splanchnopleure, from which is derived the smooth musculature of the intestinal tract. Whether or not all the striated branchial muscles are also derived from this element is not entirely certain. Between the two plates is a coelomic cavity. In other parts of the body, or in vertebrates that have long since discarded all vestige of a lateral line system the distinctiveness in origin of the dorsal from the lateroventral musculature tends to become obscured in the embryonic picture.

Joining the somites are myosepta, but the segmental character of most of the musculature has been lost in the higher vertebrates.

The initiation and early growth of muscles appear to be independent of the nerve supply, just as the eye will continue to grow for a time when transplanted to another part of the body.

In the fish it has been demonstrated that the musculature of the appendages is formed by budding of the myomeres, as explained later, and the character of the innervation suggests that in all vertebrates the limb muscles had similar derivation. In the higher forms, however, the initiation is by condensation directly from the mesoderm of the limb buds themselves. It may not be illogical to assume that in these the early budding stage has dropped out of the embryological picture; otherwise the doctrine of the continuity of vertebrate phylogeny becomes doubtful.

## GENERAL CONSIDERATIONS OF THE NERVOUS SYSTEM

The nervous system has played a far more forceful rôle in the phylogeny of vertebrates than is generally conceded, and certain of its characteristics must here be discussed.

*Branchiomeric nerves.* Although embryology appears to indicate that the central nervous system has not had a segmental or

neuromeric origin, a form somewhat simulating segmentation has been at least secondarily assumed, to conform to the myomeres. In the case of the cranial nerves this is more graphically illustrated by such protovertebrate forms as ostracoderms. In these Stensiö (1927) shows what clearly appears to have been an oral (or vestibular) branch and a second pharyngeal branch of n. trigeminus (V) succeeded by components of nn. facialis (VII), glossopharyngeus (IX), and vagus (X), all to successive branchiosepta (fig. 1). Other branches of the vagus supplied a more posterior series, successively smaller, of branchiosepta. There is every reason for assuming that this was essentially the basic plan, that the vagus nerve originally supplied one or two of the posterior gill segments, and that additional segments, with vagus innervation, were added to the end of the series as the need arose.

*Occipitospinal nerves*. It is now well known that in at least some of the members near the bottom of the vertebrate ladder there were a number of suboccipital nerves, now undeveloped in living vertebrates as indicated by their vestiges in such elasmobranchs as *Heptanchus* (Daniel, 1922). In living vertebrates of a low order, however, the motor element subsequent to the vagus is furnished by the suboccipital and first one or two of the spinal nerves, which join to form the hypobranchial nerve, supplying the trunk musculature between the coracoid and lower jaw. In the higher vertebrates this becomes the hypoglossal (XII) nerve, with root situated endocranially.

*Spinal nerves*. The true spinal nerves of vertebrates have maintained a most uniform pattern. Each body segment has a pair of dorsal sensory roots, and a pair of motor roots from the ventral part of the cord. Fundamentally the former follow the myosepta and the latter the myomeres, and so come off of the cord in alternating order. Each root, both sensory and motor, divides, sending a dorsal ramus above the lateral line and a ventral ramus below. The two roots remain distinct in the lancelet, chiefly so in the lamprey, and to some extent in various elasmobranch embryos; but in vertebrates of higher organization there is fusion of each sensory with its corresponding motor branch, the connection beginning peripherally and progressing centrally (Allen, 1917). In these

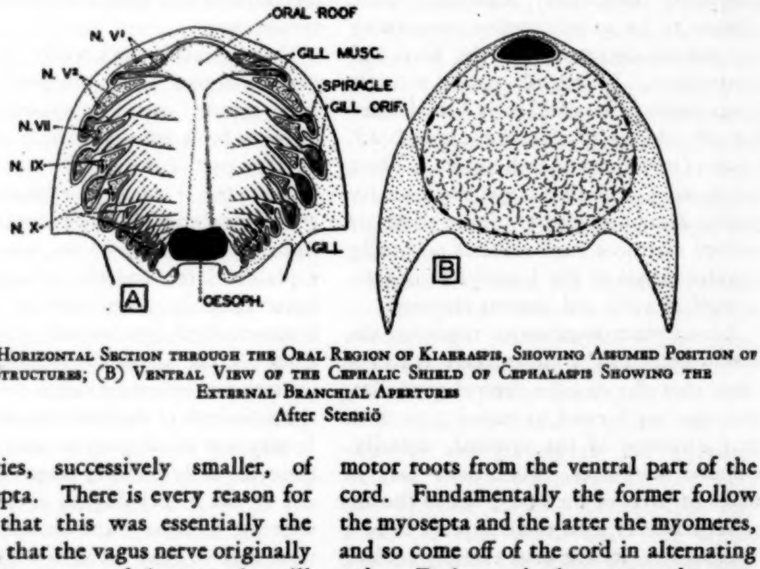


Fig. 1. (A) Horizontal Section through the Oral Region of Kiaeraspis, Showing Assumed Position of Gill Structures; (B) Ventral View of the Cephalic Shield of Cephalaspis Showing the External Branchial Apertures
After Stensiö

the result attained is that there is junction of the sensory with the motor root, and a redistribution of the fibers, so that upon each side the dorsal ramus of the spinal nerves contains both sensory and motor fibers, and the same is the case in the ventral ramus. It should be stressed that this plan of dorsal ramus above the lateral line and ventral ramus below is invariable in vertebrates. Later, in vertebrate history, there was experienced a tendency for the division of the ventral ramus into a lateral and a ventral branch, in accordance with division of muscular groups, but this was secondary.

*Nerves to appendages.* Whereas the distribution of the dorsal rami of the spinal nerves is relatively uniform, that of the ventral rami is affected by the presence of the appendages. It has been found (Howell, 1933) that the primary neural plan for the limbs is for the anterior nerve or nerves to supply a system of protractor muscle slips, for advancing the appendage, while the more caudal nerves supply a retractor muscle series, for retarding the fin. This plan of appendicular control, here called the primary basic arrangement, was not suited to an active, free-swimming vertebrate, and accordingly there was experienced a rearrangement of the muscular units, with consequent effect upon the distribution of the nerves. More specifically there was assumed a secondary basic plan, in which the protractor and retractor muscles divided into extensor and flexor elements. Thus each nerve to the appendage eventually acquired a dorsal, extensor branch and a ventral, flexor branch, simulating the lateral and ventral branches of the other trunk nerves, but independently developed. This occurred so far back in the phylogeny of the higher vertebrates that it is an invariable character of all living, terrestrial forms with limbs, at least in the higher members of which the extensor nerves of the pectoral appendage are always readily distinguishable from those of the flexor series.

*Number of brachial nerves.* The number of spinal nerves contributing to the innervation of the anterior limb is extremely variable in the lower vertebrates, there being 3 in some amphibians, reptiles and birds, and, among the fish, 55 or more in *Trygon*. Even the posterior cranial nerves may be involved in some of the skates. The number varies, of course, with the number of myotomes concerned in the formation of the appendicular musculature. In a fin with broad base the number may readily increase or decrease, but it is likely that after the base of the fin has once become constricted, following increase of motility in the axial direction, the number remains constant within very narrow limits in any particular phylum. Such constriction of the limb base gathers the nerves into a narrow area. The primitive position of the brachial nerves, and hence of the myotomes from which the brachial muscles arose, would appear to be directly posterior to the hypobranchial series. Great alteration in the position of the brachial nerves is relatively rare, but is extreme in some birds and particularly in reptiles, for as many as 76 cervical vertebrae occurred in some plesiosaurs.

*Formation of plexuses.* The morphology of plexus formation, or anastomosis of the nerves supplying the appendages, has never been fully explained, but it appears to have occurred in correlation with the crowding of muscles of plurisegmental derivation into a narrowed fin base. The less plausible of two hypotheses is based on the supposition that at one time there was a general, peripheral anastomosis between spinal nerves, such as occurs so extensively in living Holocephali, but that these connections were, in higher forms, abandoned except in the case of the ap-

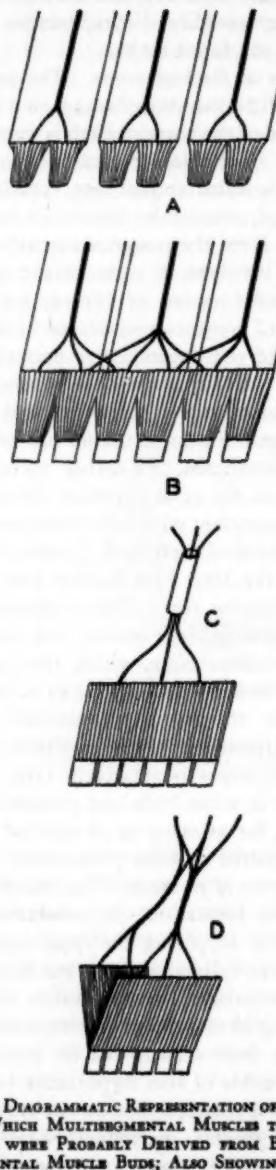


FIG. 2. DIAGRAMMATIC REPRESENTATION OF MANNER IN WHICH MULTISEGMENTAL MUSCLES TO APPENDAGES WERE PROBABLY DERIVED FROM BIPARTITE, SEGMENTAL MUSCLE BUDS; ALSO SHOWING MOTOR INNERVATION AND PROBABLE MANNER IN WHICH NERVE PLEXUSES WERE BROUGHT ABOUT THROUGH CONCENTRATION OF NERVE BRANCHES WITH INCLUSION IN A COMMON SHEATH, AND FINALLY SPLITTING OF THE MUSCLE COMPONENT

pendicular nerves, in which the crowding or plurisegmental character of the components proved favorable for the retention, or at least the proximal migration, of this feature. The more likely explanation of the formation of the limb plexuses lies in the fact that some of the appendageous muscles of relatively small size are derived from a number of myomeres. Hence the nerves supplying any one such muscle are closely grouped and parts of them may come to be enclosed in a single sheath, forming a "cord." Later a second muscle layer splits off from the first, in response to functional needs, and the nerve fibers to it will tend to split from the parent nerves in a fashion suggested in figure 2, finally resulting in a typical plexus formation.

*Reflex action.* The functioning of the nervous system is a subject entirely too complex, with many of its details too poorly understood, to discuss at length here, but a few salient facts should be mentioned.

In final analysis all action is reflex in character. The critical difference between the activities of an animal of low organization and of man lies in the fact that in the latter case the motor responses are more elaborate, complex and less predictable. In a simple type of reflex arc a sensory impulse, such as arises from pressure upon the sole of the foot, is received and transmitted by a sensory neuron to the spinal cord. It is transferred through a synapse to a short associational neuron, which in turn transmits it to a motor neuron in the ventral horn of the gray matter of the cord, from which it travels by way of an axon in the motor root to the peripheral effector (muscle), and a response results. In this particular case the receptor is proprioceptive (muscle sense) and the mechanism may be confined to half of a single body segment. Complexity may be introduced into the arc by the branch-

ing of the axon of the associational neuron, the impulse then being transmitted to a number of motor neurons at the same or at other levels, and upon the same or opposite side, of the cord.

Particularly in low forms of vertebrates if the spinal cord be isolated from the brain then the limbs still have a complete nervous equipment for the performance of the above type of action, the degree of perfection of coördination depending upon the development of associational connections. To be of practical use in progression reflex action must be of a rhythmic character, involving successively the excitation of a group of muscles effecting prime movement, and then a group of antagonist muscles for recovery, with fixation muscles contributing. The rhythm may be simple and concerned with the flexors and extensors of a single limb or may be highly complex, bringing into play not only other limbs to effect diagonal progression or simultaneous action but bending of the entire body. Whether an animal shall leap like a kangaroo, gallop or trot is determined by the organization of the reflex arcs.

Experiments in this field have been numerous but only a few instances will be cited. Snyder (1904) found that in a spinal urodele (*Batrachoseps*) walking is due to impulses originating in the periphery, such as those of stretching or of impact. I have found that in an alligator electrical stimulation of a single fore limb muscle (not its nerve, necessarily) may be sufficient for protractive and retractive movement not only of that member, but of the other limbs as well, chiefly the opposite and diagonal appendages, although this was not well coördinated. Sherrington (1920) found that in a spinal dog resting on its back, if one hind paw be pressed that leg will be flexed at hip, knee and ankle, and if the stimulus be strong the opposite limb will be extended. But if both hind paws be pressed then both limbs are simultaneously flexed and there is no trace of extension. The facility of spread from one side of the cord to the other (commissural pathways) was found to be very dissimilar at different levels, and motor mechanisms which are yoked together are (in the dog) for the most part of an asymmetrical kind, as the extensors of one side with the flexors of the other. In many animals it was found less easy to excite movement of one fore limb from the other than one hind limb from the other.

Newly initiated actions are dependent upon new connections established by associational neurones. When one learns a difficult feat of legerdemain it involves not primarily the development of muscles but the establishment of new reflex pathways, and it is probable that in certain parts of the nervous mechanism new connections can always be established through education (Edinger, 1911).

But reflex action can be instituted through various sensory pathways other than proprioceptive—by touch, temperature, pain, taste, smell, hearing, sight. The higher the animal in the scale of development the more complex are the motor responses, involving as they often do reflex pathways through the brain. Although many types of sensory impulses play on the nervous system of primitive forms the motor side of the reflex arc is poorly developed and the possible types of responses are few. Such limitations in a particular phylum must be of the utmost fundamental importance to its potentialities for progressive evolution.

In the white matter of the cord are tracts of sensory nerve fibers ascending from the different levels of the cord to the brain (afferent systems) and motor axons (corresponding efferent systems) constituting descending tracts from the brain effecting both voluntary and involuntary

action. In the lower vertebrates the former predominate, while in the higher there is an increase in the latter (e. g. development of corticospinal tracts in the Mammalia), showing the increasing importance of the brain in controlling reflex activity and the further elaboration of the motor side of the reflex arc.

*Posture.* Physiologists like to think of tone in the striated muscles as postural in quality inasmuch as it enables the organism to resist the force of gravity. A urodele must control tone in the extremities through very simple reflex arcs involving only the axis of the nervous system. In higher forms an elaborate reflex mechanism for tone control is developed in the mid-brain. This was first demonstrated by Sherrington. After decerebration of mammals there is rigidity of the extensor or anti-gravity muscles of the limbs. Curiously enough this is not true of the sloth, a mammal in which the flexor muscles normally support the body in an inverted posture. Here the flexor muscles of the limbs show increased tonus after removal of the forebrain. This postural reflex, maintained and influenced by many types of sensory stimuli, particularly proprioceptive and vestibular, controls the habitual positions which an animal maintains. Thus the horse or elephant is able to sleep standing up. Bagley and Langworthy (1926) detected the early control of tone through the mid-brain in reptiles, for they found in an alligator that extensor rigidity followed an attempt by the decerebrate animal to walk. A high type of terrestrial life is utterly impossible without an elaborate development of the anti-gravity reflex and its further differentiation through arcs involving the brain was one of the several prerequisites acquired by that group of reptiles which supposedly gave rise to the Mammalia.

*The Rolleston-Fürbringer Theory.* This hypothesis postulates that each motor nerve bears a constant relation to its corresponding myomere throughout both phylogeny and ontogeny. This is believed by the majority of present day anatomists to be a fact, although there have been a number of investigators (Cunningham, Leche, Westling, Kohlbrügge, Romer, Edgeworth, Wheeler and Adams) who consider that exceptions occur. In other words each muscle slip, no matter how far it may wander during evolution from its original position, will always be innervated by the motor nerve of its original segment. Apparent exceptions have almost always proved to have been attributable to a misunderstanding in interpreting conditions. One frequent misconception is to the effect that the numerical position of the segment by which the axon leaves the cord is the essential criterion. This need have no bearing on the matter, however, for the important point is the position of the motor nucleus, so the statement should be modified to read that the relation of a muscle to its original motor nucleus in the central nervous system remains forever constant.

*Law of neurobiotaxis.* This law, advanced by Kappers, postulates that in the course of phylogeny the neurons of the motor nuclei tend to migrate in the direction from which they habitually receive their stimuli. This has been pretty well demonstrated for the brain but very little work with this in mind has been done on the spinal cord.

*Possible migration of the motor nuclei in the cord.* The extensor muscles, say, of the arm in man arise usually from sp. nn. 5 to 8, and the motor nuclei of these nerves (axillary and radial) lie in the adjoining part of the cord. But nerves to other groups of muscles (flexors) arise from these roots as well, so the nuclei of different muscles and muscle groups are packed within the ven-

tral horn of gray matter in aggregations. Whether the latter are always of a concentrated character or at times are esssentially diffuse or discrete is a matter yet to be determined. At least the position of the nuclei is remarkably constant within the class Mammalia, but there is some variation, both ordinal and individual. Thus, although the radial nerve usually arises from the sixth to eighth cervical nerves, occasionally it comes from one root higher or one lower. In other words it seems that its motor nucleus occasionally wanders fractionally either above or below its usual position. Although it is believed that the difference in the brachial neural plan of a low urodele, for instance, and a mammal is attributable largely to basal ancestral dissimilarity, it is not unlikely that it is partly due to the migration of the motor nuclei. Thus it is conceivable that a muscle slip now innervated by the fifth cervical nerve in a mammal may have been derived from one segment higher or (less likely) lower in an ancestral form.

*Plurisegmental innervation*. Not only may a single small muscle have been derived from a number of myomeres, as elaborated elsewhere, but it has been demonstrated that in fish this may involve a variably slight degree of overlap of the segmental elements (Braus, 1909; Goodrich, 1910; Müller, 1911). Some (Goodrich, 1930) have considered that this involves not a wandering of a nerve to muscle fibers of adjoining segments but rather to a migration of fibers from their own to neighboring myomeres. In seeming controversion of this interpretation, however, is the fact that in mammals and in the frog at least the same muscle fiber may be supplied by motor twigs from two, or even three adjoining neuromeres (in Hines, 1927). It has never been explained how this could be so and the peripheral nervous system still retain its astonishing conservativeness in other respects throughout so many millions of years.

### GENERAL CONSIDERATIONS OF THE MUSCULAR SYSTEM

Many of the basic features of the muscles have been touched upon in the last section, but there are additional details needing consideration.

*Somatic and branchial musculature*. All striated musculature (except cardiac) is divisible into these two groups, although the division can not be made on hard and fast lines except on the basis of innervation. For the most part somatic muscles have muscle spindles (for proprioceptive purposes) while branchial muscles do not; but there are exceptions, for these have been found in the m. pterygoideus externus and the m. masseter of the rabbit (Cipollone, 1897) and in the latter muscle of the fetal pig (Cuajunco, in Hines, 1927). They have not been found in the extrinsic eye muscles of the dog, fox, cat, hare, rat, horse, and domestic pig (Cilimbaris, 1910). Occasionally even the criterion of gross innervation fails definitely to distinguish somatic from visceral muscles (spinal innervation of m. trapezius) and recourse must be had to experiments involving degeneration of the motor nuclei. In general only somatic muscles are considered to have segmental innervation, but actually the branchial musculature also conforms basically to a segmental arrangement. The latter, primarily concerned with the pharyngeal mechanism, becomes partially subcutaneous and invades the field of somatic musculature. The basic arrangement of the branchiomeric division has already been mentioned.

*Basic segmental features*. The original plan of vertebrate trunk musculature, well illustrated by cyclostomes, involves a series of segmental muscles each of which is

separated from the muscles of adjoining segments by myocommata or myosepta. The axially directed muscle fibers of each segment are basically divided into a dorsal division, above the lateral line on either side of the midline, and a continuous latero-ventral division below; this constitutes the primary muscular plan. It is a primitive scheme, suited to a low vertebrate that can bend with equal facility in any direction—the essentially vermiform type of control.

In this plan the myosepta are virtually transverse and usually gently curved. Unlike the situation in mammals, most of

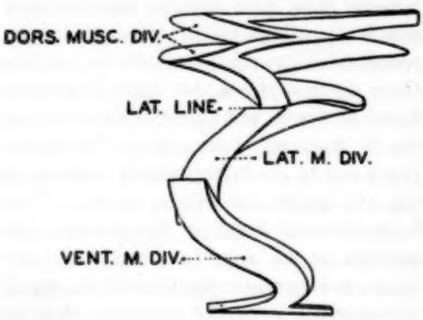


FIG. 3. MODEL OF MYOMERE OF A SELACHIAN (SQUALUS)
Modified from Langelaan and Daniel

whose muscles have one end solidly anchored on bone, in the primitive state the fibers at both ends are attached to yielding connective tissue. Accordingly there was originally a tendency for some of the groups of fibers to pull certain parts of the myosepta in a forward and others in a backward direction, as a result of specialized action of the groups concerned. This would have a contortional effect upon the myosepta, and in consequence some parts would have an anterior and others a posterior inclination, as suggested in the given diagram of a myomere of a shark (fig. 3). Presumably the swifter the fish (i.e. the stronger the muscle action) the more tortuous the pattern of the myosepta.

*Adoption of trunk muscle divisions.* A result of the latter is the secondary basic plan of vertebrate musculature involving (a) the original dorsal element above the lateral line, an extensor of the trunk, thick because located near the notochord and having short leverage, (b) a ventral component, split off from the original latero-ventral musculature to form a flexor of the trunk, tending to be thinner than the extensor for the reason that it is located farther from the notochord and hence with longer leverage, and (c) a lateral muscle division below the lateral line on either side for horizontal flexure of the body. The development of this plan has been in response to the need, as evolution progressed, for movement in restricted planes, extension and flexion, abduction and adduction. The divisions most probably took place at the points where the angles formed by the alteration in the direction of the myosepta were most acute; actually such changes in the myosepta must have been the first indications of future muscular divisions. Variations in the relative size of the ventral and lateral divisions occur in accordance with the functional stimuli to which they have been subjected. And future lesser, or tertiary, divisions occurred at the point where there were minor flexures of the myosepta.

*Basic plan of peripheral musculature.* As explained in more detail in future sections the initiation of the fins is believed to have involved development of segmental, dermal scutes, to each of which was attached a muscle slip (fig. 4). As these scutes lengthened and a fin-like structure took form, the controlling musculature first aligned itself into a posterior, prime mover group, for retraction, and an anterior, antagonist group for recovery or protraction. This was the primary plan

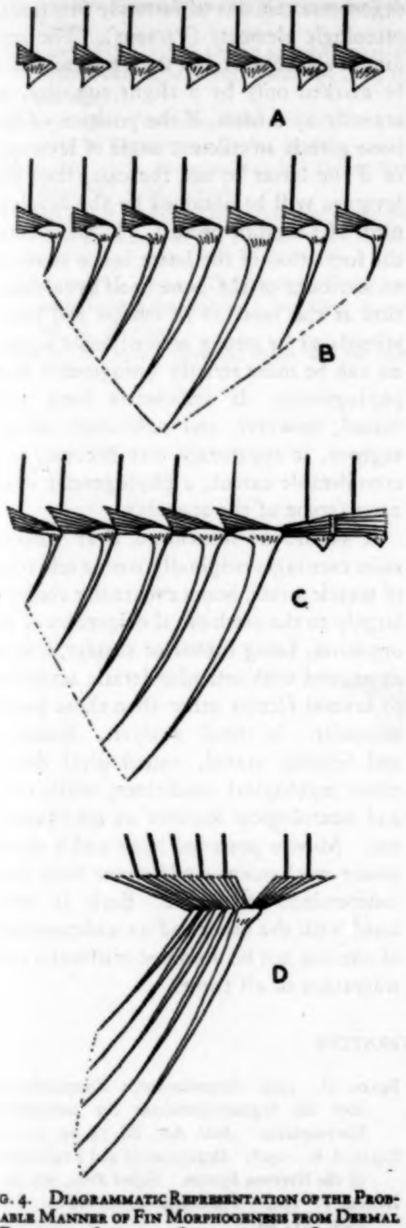


FIG. 4. DIAGRAMMATIC REPRESENTATION OF THE PROBABLE MANNER OF FIN MORPHOGENESIS FROM DERMAL ELEMENTS, SHOWING CONCRETION OF SEGMENTAL MUSCLE COMPONENTS AND THEIR MOTOR INNERVATION

and was followed in forms having the appendages composed of many segments (Selachii) by the stringing together of the individual muscle slips, as the base of the fin became contracted, into longitudinal series. The secondary plan involved a regrouping of the original protractor-retractor elements into extensor-flexor, elevator-depressor, or abductor-adductor musculature. This could be done according to any one of several schemes. All myomeres might contribute equally to the dorsal and ventral groups of fin musculature, as appears to have been the case in many teleosts, the anteriormost brachial muscle components may contribute to the formation of the cranial part of the ventral group only, as apparently is the case in *Polypterus* and in more exaggerated degree in elasmobranchs, or there might be other schemes. From this point on, however, the muscular differentiation varied in pattern with the diversity of the stimuli encountered in different groups of fish, so that variation is great.

## GENERAL CONSIDERATIONS OF THE SKELETAL SYSTEM

As already stated bones are either membranous (dermal) or cartilaginous in derivation. The two occasionally fuse, the cartilage then showing a tendency to disappear through absorption. Cartilage (and therefore bone) can occur wherever there is the proper stimulus, and this is usually muscle stress, in myosepta and between muscle groups. Whether cartilage remains in that state or becomes bone depends upon several factors, some of which are obscure. On the whole it is usual for cartilage to predominate in extremely low vertebrates and in those sorts in which the muscle action is weak or distributed through broad attachments (urodeles). There is much group difference, however, and there is no reason clear to us why a

shark should be largely cartilaginous while such teleosts as the shad should develop slender bones in practically every conceivable situation.

As far as one can see cartilage is the same whether occurring as a costal, appendageous, or branchial element, not a primary necessity but something developed where need arises, just as connective tissue occurs in a variety of situations. It may develop a joint, enclosed by an articular capsule, whenever there is need for one, the new element being preceded by a process projected from the old, and such cartilages (or bones), at least elsewhere than the skull, usually constitute the anchorage of functional groups of muscles. Occasionally, however, there occurs cartilage purely for stiffening purposes (extrabranchials). A cartilage (or bone) will persist as long as it functions as an anchorage for muscles. It will have a movable joint for attachment to other cartilages as long as motility is advantageous, but if structural strength become a desideratum then the movable joint will eventually be replaced by an immovable suture.

A bone constituting a single phylogenetic element will usually have but a single primary center of ossification. Secondary centers in the nature of epiphyses with sutural attachment may develop from the transmission of weight (pressure-epiphyses) or where muscles of great power have restricted attachment (traction-epiphyses). A third type (atavistic epiphyses) are the degenerate remains of formerly functional osteologic elements (Parsons). The tendinous attachment of a strong muscle may be marked only by a slight rugosity, or actually by a fossa, if the position of the bone give it an efficient angle of leverage, or if the latter be not the case, then the leverage will be obtained by the development of a crest or process. In many cases the formation of the latter is not so much an attribute of the bone itself as calcification at the junction of tendon and bone, stimulated by strong action; hence a process can be more strictly ontogenetic than phylogenetic. If sufficiently long continued, however, and sufficiently advantageous, it apparently may become, to a considerable extent, a phylogenetic character (spine of the scapula).

It should be mentioned that whereas most cartilages originally were a reflection of muscle stress, bones eventually conform largely to the mechanical exigencies of the organism, being robust or slender, long or short, and with articular details according to several factors other than those purely muscular. In final analysis, however, and broadly stated, osteological details reflect myological conditions, while these and neurological features are interdependent. Muscle preceded bone and a neural motor mechanism could never have been independent of muscle. Each is correlated with the other and an understanding of one can not be obtained without a consideration of all three.

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# ON THE RATE OF OXYGEN CONSUMPTION BY TISSUES AND LOWER ORGANISMS AS A FUNCTION OF OXYGEN TENSION

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## I

THE information dealing with the rates of $O_2$-consumption by tissues and lower organisms as function of $O_2$ tension in the surrounding media was summarized up to 1914 by Warburg (1914). Since then numerous data of a quantitative nature have been obtained, but as far as the writer is aware, no attempt to summarize the recent, perhaps more important, measurements has been made save that by Hyman (1929), who was interested in the matter through phylogenetic and ecological considerations. In view of the theoretical equations developed in recent years attempting to describe the rate of $O_2$-consumption as a function of $O_2$ tension (Warburg and Kubowitz, 1929; Shibata and Tamiya, 1930; Gerard, 1931; Roughton, 1932), and in view of the fact that the existing data are presented in very many diverse ways so that they are not readily comparable, it was deemed desirable that a quantitative summary and interpretations of the more recent work on the subject be made.

While it is undoubtedly true that in many cases the rate of $O_2$-consumption is independent of $O_2$ tension for the range tested, it is equally true that in a great number of cases definite relations exist between the two; and the possibility still remains that if the lower limits of the ranges of $O_2$ tension tested were extended, provided no irreversible changes occur in the respiring material, the rates which are considered independent of $O_2$ tension in many forms may prove to be dependent. In this account, we shall limit ourselves only to the cases where a definite relation exists between the two.

With a very few exceptions where the rate of $O_2$-consumption is directly proportional to $O_2$ tension, the type of relation obtained is hyperbolic; that is, the rate of $O_2$-consumption varies rapidly with $O_2$ tension at low concentrations, less so at higher concentrations, and a point is eventually reached where further increase in $O_2$ tension produces little or no effect on the rate of $O_2$-consumption, the tension at which the curve approaches saturation being the "critical pressure."

In the following treatment, all data (with one exception) were obtained from the numerical determinations given in the original articles, and all terms were converted into comparable units wherever possible. Thus the expression of $O_2$ tension in terms of percentage, in cc. $O_2$ per cc. water, cc. thiosulfate per cc. water, per cent saturation, etc., have all been converted into mm. Hg. In many cases the correction for vapor pressure was not specified, and it was assumed that such a correction was made. The error so introduced is not very serious, however. Instead of a percentage, the rate of $O_2$-consumption $A$, is expressed in terms of cc. of

$O_2$ consumed per hour per gram dry weight. In case dry weight was not given, "fresh weight" or "individual" is substituted. $A$ is used in this paper as a general term designating the rate of consumption of $O_2$ expressed in cc. $O_2$ per hr. per gm. dry weight or fresh weight, or per individual. It therefore includes the $Q_{O_2}$ of Warburg. $A_0$ is the maximum rate. In the experiments where the respiring material was kept in a closed container, and was allowed to reduce the $O_2$ tension in the surrounding medium by virtue of its own respiratory activity, and the amount of $O_2$ left in the medium was plotted against time, the data were recalculated in terms of amount of $O_2$ consumed as a function of $O_2$ pressure.

## II

While it is not the purpose of the present account to review the various methods used in ascertaining the rate of $O_2$-consumption as a function of $O_2$ tension, a brief résumé is not out of place.

In general, the technics may be divided into two types. One of these is that of placing the respiring material in an enclosed system with a known initial concentration of $O_2$. The rate of consumption of $O_2$ is then followed either manometrically or by titrating the surrounding water as the tension in the system is reduced through the activity of the respiring material. While this type of method has its advantage over the one to be discussed below because the data in this case are obtained on the same material and therefore are not susceptible to variations arising from using different samples, it has many disadvantages. Since in such experiments the duration is usually over a period of several hours, the accumulation of waste material, especially of $CO_2$, change of pH of medium, lack of adequate stirring, and "adaptation," among others, may cause errors in measurements.

The second type of technic consists in subjecting the respiring materials for short intervals to gas mixtures of various $O_2$ tensions and comparing the rates with that of the control experiment at a given $O_2$ tension (usually that of the air). While this type of technic is relatively free from the objections mentioned in connection with the first type, it is not entirely satisfactory since the rate of $O_2$-consumption by different samples of material may not be strictly comparable, and since the $O_2$ tension in the surrounding medium is the mean tension from the beginning of the experiment to the end. Where the amount of $O_2$ consumed during the experiment is relatively large, as is necessarily the case where direct analysis of the gas is made, the uncertainty in expressing the $O_2$ tension is considerable.

In both cases, save where the $CO_2$ produced during the experiment was removed by suitable means, serious errors may arise from the effect of $CO_2$ on the rate of $O_2$-consumption (cf. Root, 1930). And except where the respiring materials are suspended in a gas medium, errors may result from insufficient stirring of the water surrounding them. The new technic of Warburg and Kubowitz (1929) was designed especially with the last point in view, but the high speed at which the respirometers are rotated may introduce additional complications due to possible injury to the respiring materials.

The relative merits of the methods used in the various accounts should be kept in mind in going through the discussions given in the following sections.

## III

Without going into theoretical considerations, we shall, for convenience and conciseness, treat the relationship existing between the rate of $O_2$-consumption and $O_2$ tension by the equation:

$$A = \frac{P}{K_1 + K_2P} \qquad (1)$$

in which $A$ represents cc. $O_2$ consumed per hour per gram dry weight (or units pro-

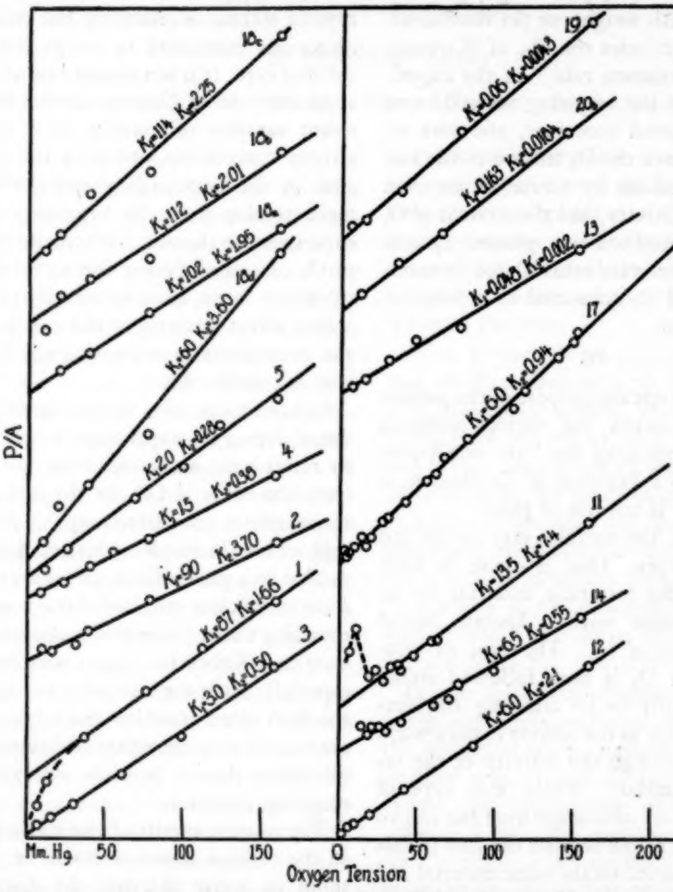


FIG. 1. $A/P$ IN ARBITRARY UNITS IS PLOTTED AGAINST $P$, THE PARTIAL PRESSURE (CONCENTRATION) OF OXYGEN, IN MM. HG., $A$ BEING THE RATE OF $O_2$-CONSUMPTION

The intercepts ($K_1$) and the slopes ($K_2$) of the lines are given along with the curves. The numbers attached to each curve refer to the material used, as listed in Table 1.

portional thereto); $P$, the $O_2$ tension in mm. Hg.; and $K_1$ and $K_2$ are constants.

This equation not only takes into consideration the hyperbolic type of relation, but can be made to express all three empirically determined types of relationship existing between $O_2$-consumption and $O_2$ tension. For, if $K_1$ is comparatively much larger than $K_2P$, $A$ is approximately equal to $P/K_1$ which is descriptive of the small number of data where the rate of $O_2$-consumption is directly proportional to $O_2$ tension. Conversely if $K_1$ is very small compared to $K_2P$, $A$ may be considered a constant, and this is descriptive of the cases where the rate of $O_2$-consumption is apparently independent of $O_2$ tension.

If equation (1) is a fair description of the relation existing between $A$ and $P$, by

plotting $P/A$ against $P$ one should obtain a straight line with $K_1$ as its intercept and $K_2$ as the slope. Twenty-four different sets of data are so plotted in Figs. 1 and 2. attached to each line is the index of the object used, which is the same as that given throughout the text and in Table 1. The constants, $K_1$ (the intercept) and $K_2$ (the

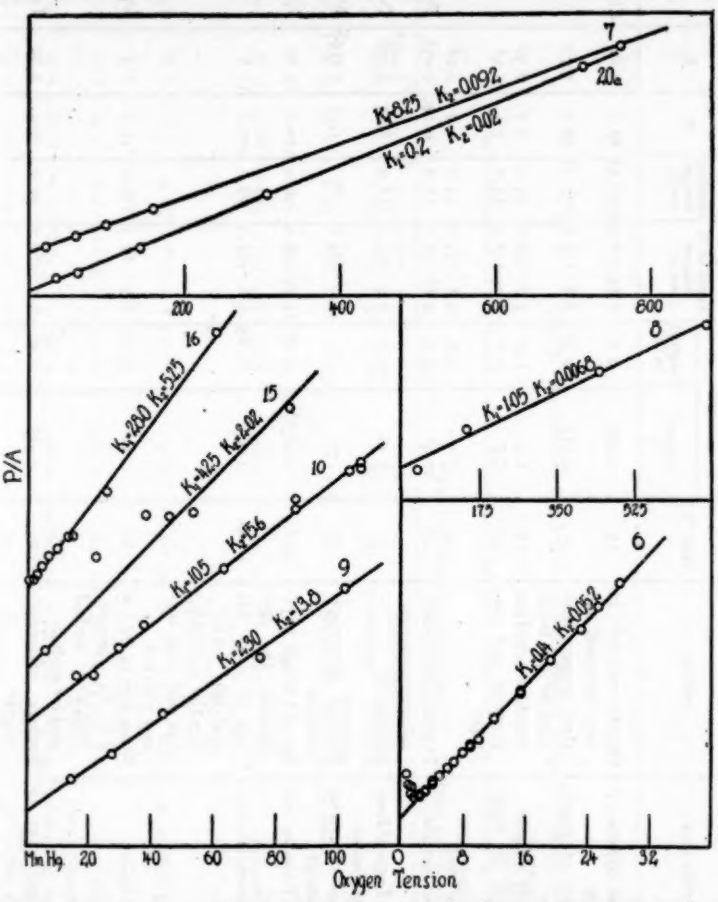


FIG. 2. $A/P$ IN ARBITRARY UNITS IS PLOTTED AGAINST $P$, THE PARTIAL PRESSURE (CONCENTRATION) OF OXYGEN, IN MM. HG., $A$ BEING THE RATE OF $O_2$-CONSUMPTION

The intercepts ($K_1$) and the slopes ($K_2$) of the lines are given along with the curves. The numbers attached to each curve refer to the material used, as listed in Table 1. The points for curve 15 were obtained at temperatures ranging from 16.5 to 23°. (See text.)

All the points are taken from original data except those for curve 16, where the points were taken from curve $D$ in Fig. 3 of Dolk and Van der Paauw (1929). The number slope), are also given along the lines. Thus Figs. 1 and 2 are not only a graphic presentation of the validity of the application of equation (1), but are also a dia-

TABLE 1

*A summary of the essential points for the accounts mentioned in section III. The value of A for any value of P may be obtained through the relation* $A = P/(K_1 + K_2P)$

| | MATERIAL USED | METHOD | TEMP. °C. | $A_0$ Observed | $A_0$ Calculated $(1/K_2)$ | RANGE OF $O_2$ TENSION mm.Hg. | CRITICAL TENSION mm.Hg. | $K_1$ | $K_2$ | AUTHOR |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Leaves of *Hypnum triquetrum* | Objects enclosed in jars: evacuated and then proper mixtures of gas introduced. Respired gas extracted and analyzed | 25 | 0.460 | 0.6 | 7.22–158.5 | (158.5) | 87.0 | 1.66 | Chevillard, Hamon, Meyer and Plantefol (1930) |
| 2 | Fragments of potato tubers | | 25 | 0.237 | 0.27 | 8.8 –159.0 | (159.0) | 90.0 | 3.70 | |
| 3 | Fragments of turnip | | 25 | 1.9 | 2.0 | 7.0 –158.0 | 55.0 | 3.0 | 0.50 | |
| 4 | Fragments of mushroom | | 20 | 2.6 | 2.6 | 8.05–158.0 | 55.0 | 1.5 | 0.38 | |
| 5 | Leaves of cress | | 20 | 3.5 | 3.6 | 10.8 –159.0 | 55.0 | 2.0 | 0.28 | |
| 6 | Luminous bacteria | Thunberg-Winterstein microrespirometers | 21[1] | 17.4[1] | 19.2 | 0.68– 28.1 | 22.8 | 0.140 | 0.052 | Shoup (1929–30) |
| 7 | Fertilized eggs of *Paracentrotus lividus* | Manometric | — | 9.56 | 11.0 | 0.0 –760.0 | 228.0 | 8.25 | 0.092 | Drastich (1927) |
| 8 | Oxidation of cystine on charcoal | Warburg microrespirometer | 40 | — | — | 36.0 –684.0 | — | 1.05 | 0.0068 | Warburg (1928) |
| 9 | Puffer (*Tetraodon maculatus*) | Ege and Krogh respirometer. $O_2$ content of water was analyzed with Winkler method before and after contact with fish | 20–21 | 0.0636[4] | 0.07 | 14.35–102.0 | 100.0 | 230.0 | 13.80 | Hall (1929) |
| 10 | Scup (*Stenotomus chrysops*) | | 20–21 | 0.063[4] | 0.065 | 15.8 –107.0 | 70.0 | 105.0 | 15.6 | |
| 11 | *Planaria agilis* | Worm kept in $H_2O$ of diff. $O_2$ tension. Winkler method used for analysis | 20 | 0.12[4] | 0.135 | 6.5 – 62.5 | 159.0 | 135.0 | 7.4 | Lund (1921) |
| 12 | Unfertilized eggs of *Arbacia punctulata* | Warburg microrespirometer | 25 | 0.485 | 0.48 | 2.0 –160.0 | 40.0 | 6.0 | 2.10 | Tang (1931) |
| 13 | *Azotobacter chroococcum* | New method of Warburg and Kubowitz | 10 | 435.0 | 500.0 | 7.6 –152.0 | 110.0 | 0.048 | 0.002 | Meyerhof and Schulz (1932) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 14 | Fertilized eggs of *Arbacia punctulata* | Warburg microrespirometer | 25 | 1.7 | 1.8 | 13.0 –155.0 | 50.0 | 6.5 | 0.55 | Tang and Gerard (1932) |
| 15 | Fragments of *Chironomus thummi* larvae | Warburg microrespirometer | 16.5–23.0 | 0.400[4] | 0.495 | 6.1 – 83.5 | 70.0 | 42.5 | 2.02 | Harnish (1930) |
| 16 | Earthworm | Modified Krogh: worm supported in gas atmosphere | 25 | 0.178[5] | 0.190 | 0.0 –159.0 | 19.0 | 28.0 | 5.25 | Dolk and Van der Paauw (1929) |
| 17 | Fertilized eggs of *Arbacia* | Eggs rotated in tonometer with proper gas mixture. Gas analyzed afterwards | 18.2–20.2 | 1.0[3] | 1.04 | 3.0 –224.4 | 20.0 | 6.0 | 0.94 | Amberson (1928) |
| 18 | *Dixippus morosus* | Krogh microrespirometer | 17 | 0.314[2,5] | 0.40 | 9.5 –160.0 | 159.0 | 94.0[2] | 2.45[2] | von Buddenbrock and von Rohr (1923) |
| 19 | Termites (*Termopsis nevadensis*) | Warburg microrespirometer | 20 | 213.0[6] | 230.0 | 6.1 –760.0 | 40.0 | 0.06 | 0.0043 | Cook (1932) |
| 20*a* | Yeast | Warburg differential microrespirometer | 37 | 48.0 | 50.0 | 36.0 –712.0 | 100.0 | 0.2 | 0.02 | Cook (1930–31) |
| *b* | | | 37 | 48.0 | 55.0 | 11.5 –144.0 | 100.0 | 0.163 | 0.0184 | |

[1] Temperature and $A_0$ not specified. Figures taken from Harvey (1928).
[2] Average of 4 experiments.
[3] The value of 1.0 was arrived at from the data of Tang and Gerard where $A_0 = 1.7$ at 25° ($\mu$ assumed to be 16,000).
[4] cc./hr./gm. fresh weight.
[5] cc./hr./gm./individual.
[6] The value of 213 is reduced from the average value of 805 at 35° ($\mu$ assumed to be 16,000).

grammatic summary of the great majority of the recent quantitative data relating oxygen tension and rate of $O_2$-consumption by tissues and by lower organisms. A glance at these figures and Table 1 reveals the variety of materials studied, the ranges of $O_2$ tension used, and the constants for the different materials.

The set of data labelled from 1 to 5 are taken from Chevillard, Hamon, Meyer, and Plantefol (1930). These workers subjected leaves of *Hypnum* moss (1), fragments of potato (2), turnip (3), and mushroom (4), and leaves of cress (5) to chambers containing mixtures of gases the $O_2$-concentration of which varied from 7.0 to 159 mm. Hg. After a period in a thermostat kept at 20 or 25°, the gases were extracted from the chambers and analyzed. They found that a reduction in $O_2$ tension affects the rate of $CO_2$ production as well as $O_2$-consumption; but the two are affected differently in such a manner that the ratio of $CO_2$ production to $O_2$-consumption (Respiratory Quotient) is raised as the concentration of $O_2$ is lowered. The experiments were not performed beyond a partial pressure of 159 mm. Hg., and in the case of *Hypnum* and potato the critical concentration is evidently beyond the highest pressure tested. For the rest, the critical pressure is at about 58 mm. Hg. The slight deviation of the points at the low pressures in Curve 1 is probably due to technical errors.

In his study of the rate of oxygen consumption of the luminous bacteria as a function of oxygen tension, Shoup (1929–30) concluded that beyond a critical pressure of 22.8 mm. Hg. the rate of $O_2$-consumption remains unaltered as the oxygen tension in the medium is changed. No mention was made in the paper of the temperature nor of the absolute rate. In Table 1, the temperature and $A_0$ were taken from Harvey (1928). Shoup is of the opinion that his curve is similar to that for adsorption of gases on solid surface and that the mode of action of $O_2$ with the respiratory enzyme of the bacteria is the same as that of surface catalysis in heterogeneous chemical systems. His data are plotted in graph 6. It is seen that a straight line fits all except the points below a pressure of about 1.5 mm., where deviation occurs. In view of the uncertainties of measurements at such small concentrations of oxygen, too much significance need not be attached to this apparent deviation.

In connection with the study of the mechanism of fertilization, Drastich (1927) made some observations on the relation of oxygen tension and the rate of oxygen consumption in the fertilized eggs of *Paracentrotus lividus*, using the manometric method, without mentioning the temperature of the experiment. The points are plotted on the curve numbered 7.

Curve no. 8 is for the catalytic oxidation of cystine on charcoal surface at 40° as measured by Warburg and Negelein (1921). The points were taken during the first 21 minutes of reaction. This set of data was plotted here merely for comparison between chemical catalysis and biological oxidations. It is noticed that like the data for biological oxidations, the points can be fitted with the same equation.

Hall (1929) studied the rates of oxygen consumption of three types of fish: *Tetraodon maculatus* (Puffer), *Stenotomus chrysops* (Scup), and *Opsanus tau* (Toadfish). The procedure consisted in ascertaining the $O_2$ content of the water, with Winkler titration, before and after passing through the tank containing the fish. While he found a hyperbolic relation in the first two cases (curves 9 and 10), a straight line function was obtained with the toadfish. He correlated the findings with hemoglobin content and the speed of movement of the fish. The toadfish, which is the most

sluggish and has the least amount of hemoglobin, has not only the lowest absolute rate of $O_2$-consumption among the three, but the rate is also directly proportional to oxygen tension up to 100 mm. Hg.

Curve 11 is obtained with *Planaria agilis* by Lund (1918) using the Winkler method. Instead of having the worms in a closed container and allowing the $O_2$ tension of the medium to decrease with time as is usually done with the Winkler technic, the animals were subjected to samples of water of varied $O_2$ tension up to 410 mm. Hg. The deviation of the points at lower pressures from the straight line is probably due to errors in measurement.

Tang (1931) and Tang and Gerard (1932) secured data for the oxygen consumption-$O_2$ tension relationship in the fertilized (14) and unfertilized (12) eggs of *Arbacia*. In both cases they found a hyperbolic relationship, with critical tensions occurring at 50 and 40 mm. Hg. respectively. Unpublished data obtained by the same authors with cytolyzed eggs show that the rate for cytolyzed eggs, as that of the intact ones, bears a hyperbolic relationship to $O_2$ tension.

By the use of the new method of Warburg and Kubowitz (1929), where the respirometer was shaken at 540 oscillations per minute so that diffusion through the liquid medium is not a factor, and by the use of cells (*Azotobacter*) the diameter of which is such that diffusion through tissue is not a source of error, Meyerhof and Schulz (1932) demonstrated a hyperbolic relation between the rate of $O_2$-consumption and $O_2$ tension, with a critical pressure of 110 mm. (curve 13). These authors are of the opinion that at lower $O_2$ tensions the "respiratory ferment" is not entirely saturated but that part is dissociated, and the hyperbolic curve obtained is to be expected from the dissociation of the "ferment."

Curve 15 represents the data obtained by Harnish (1930) with finely divided fragments of larvæ of *Chironomus thummi*. This author had previously found that the rate of $O_2$-consumption of the intact animal was dependent on the oxygen tension of the surrounding medium. The experiment with fragments of the larvæ was planned to show that the relationship is a real one, and is not an artefact due to insufficient diffusion of $O_2$ through the tissues. The temperatures of the individual experiments varied from 16.5° to 23.0°, hence the data are not as exact as desired, and the points are not expected to fall on a straight line; the data are given here merely to show that insufficient diffusion of $O_2$ through the tissue is not a factor in this case and, like many others, a hyperbolic relationship still exists between $O_2$ tension and the rate of $O_2$-consumption.

Dolk and Van der Paauw (1929) studied the rate of $O_2$-consumption as a function of $O_2$-tension in the normal as well as in CO-treated earthworms. They found that in the normal worm, the part of the curve below the critical tension of 19 mm. Hg. is similar to the dissociation curve of hemoglobin. With CO-treated worms, the critical tension came at 57 mm. Hg. Their opinion is that hemoglobin in the blood of the worm plays an important rôle below the critical tension. Attempts to transpose the figures in their table were not successful and the points for curve 16 were taken from curve D, Fig. 3, of their paper.

Amberson (1928) studied the relation of oxygen tension and the rate of respiration in *Paramaecium* and the fertilized eggs of *Arbacia* by subjecting equal amounts of the cells in tonometers which were partially filled with water in equilibrium with air and with $O_2$-$N_2$ mixtures of varied proportions. After several hours during which the tonometers were in constant rotation, the gases were extracted and

analyzed. Although the data obtained with *Paramaecium* showed a slightly decreased rate of respiration with decreasing $O_2$ tension, the lower limit of the experiment was too high to bring to light the true relationship which may exist between the two. The data on the fertilized *Arbacia* eggs are more comprehensive, and it was found that while above the critical pressure of 20 mm. Hg. the rate of respiration is independent of $O_2$ tension, below that pressure, the rate falls rapidly with falling tension. His data seem to indicate that as in the case of plant tissues, the R. Q. increases with falling oxygen tension. Since the absolute maximum rate was not given, the value of 1.7 found by Tang and Gerard (1932) at 25° was reduced to 1.0 at 18° (assuming a $\mu$ of 16,000) and was adopted as a rough approximation. $K_1$ and $K_2$ were calculated on this basis.

Von Buddenbrock and von Rohr (1923) observed the rate of respiration of *Dixippus* in groups of three or more. The rate was studied as a function of temperature, $O_2$ tension, and $CO_2$ concentration and of the combination of the first two factors. Four sets of data with three insects each were obtained at different $O_2$ tensions and these are plotted as 18 *a*, *b*, *c*, and *d* in Fig. 1. The constants as well as the critical oxygen pressure of three sets (18 *a*, *b*, and *c*) agree fairly closely, while the fourth group (18 *d*) gave constants which are slightly different, but which are of the same order of magnitude as those found in the first three sets. The averaged values are given in Table 1.

Using yeast as material, Cook (1931) studied the variation in the rate of respiration and fermentation when the partial as well as the total pressure of $O_2$ were altered. It was found that while the rate of $O_2$-consumption is affected in both cases, the effects are not identical: a reduction of pressure as such induced a much greater change in rate of $O_2$-consumption than changing the concentration of $O_2$. Such a state of affairs was not found in the case of fructose. Only the two sets of data deal ing with the effect of changing of the partial pressure of $O_2$ on the $O_2$-consumption rate of yeast are given in the Figures (20*a* and 20*b*).

In a more recent paper, Cook (1932) ascertained the rate of $O_2$-consumption of termites (*Termopsis nevadensis*) in the range of partial pressure of $O_2$ between 6.1 and 760 mm. Hg., by means of the Warburg technic. The rate was found to be practically independent of $O_2$ tension down to 40 mm. Hg., when the curve fell rapidly with decreasing $O_2$ tension. The average absolute rate of oxygen consumption for 10 groups of 12 individuals each is 805 cc. $O_2$ per gm. per hr., at 35°. But the $O_2$ tension vs. $O_2$-consumption relation was obtained at 20°, and only the percentages are given. Assuming a temperature characteristic ($\mu$) of 16,000, the rate at 20° in air may be taken as 213 cc. per gm. per hr. $P/A$ is then calculated on this basis and the constants in Table 1 are computed accordingly. The points, with the exception of that taken at 760 mm. Hg. where a slight injurious effect was apparent, are plotted as curve 19.

The essential points in the accounts mentioned above are summarized in Table 1.

## IV

In a very few cases, the data show that the rate of $O_2$-consumption is a linear function of $O_2$ tension and cannot be treated in the above manner. They are therefore given separately.

Amberson, Mayerson, and Scott (1924–25) studied the rate of $O_2$-consumption of the lobster (*Homarus americanus*) and *Nereis virens* using the Winkler method and allowing the animals to reduce the $O_2$ tension of the water through their own re-

spiratory activities. These authors obtained a straight line relation between log of $O_2$ tension in water and time, hence a linear relationship between the rate of $O_2$-consumption and $O_2$ tension from about 760 to about 76 mm. Hg. ("full" and "10 per cent saturation" respectively). It is to be remembered that while a single animal was used in the case of the lobster, 82 animals were used for the experiment with *Nereis*.

Using the same technic as that of Amberson *et al.*, Chen (1930) claimed to have found a linear relationship between $O_2$ tension and the rate of $O_2$-consumption in the Chinese freshwater crab (*Eriocheis sinensis*). This is true only for the range of 160 to 720 mm. Hg. ("21" and "95 per cent saturation" respectively). The rate fell rapidly and irregularly below 160 mm. Hg. The same may be said of the results of Nomura (1926) obtained on *Caudina chilensis* between the partial $O_2$ pressures of 20 and 133 mm. Hg.

Hall (1929) found a linear relationship between the rate of $O_2$-consumption and the $O_2$ tension of the surrounding water in the Toadfish, while such is not the case in the Scup and Puffer as mentioned above. The difference was attributed to the sluggishness and the lower hemoglobin content of the Toadfish.

Tamiya (1929) obtained a direct relationship between the rate of $O_2$-consumption and $O_2$ tension in *Aspergillus oryzae* from 0 to 509 mm. beyond which the relation fails to hold. An explanation of this observation was attempted later by Shibata and Tamiya (1930) in terms of the absence of cytochrome, and the influence of diffusion.

While linear relationship between the rate of $O_2$-consumption and $O_2$ tension of the media in the aforementioned cases may be entirely correct, technical errors such as insufficient rate of diffusion of the gas through the liquid medium as well as through the tissues, among other factors, must not be overlooked, especially in the experiments where such animals as the crab and lobster were used.

## V

It is evident from the data collected that the hyperbolic equation

$$A = \frac{P}{K_1 + K_2P}$$

holds true for most of the measurements dealing with the rate of $O_2$-consumption as a function of $O_2$ tension. This type of equation was first derived for the $O_2$-dissociation curve of hemoglobin by Hüfner and by Hill (see Barcroft, 1928) and for that of hemocyanin by Stedman and Stedman (1928), and Redfield (1930). Warburg and Kubowitz (1929), and later Shibata and Tamiya (1930), and Gerard (1931), arrived at a similar type of equation for cell respiration, based upon the assumption that the following reactions take place when $O_2$ is being consumed by the respiring material through the catalytic action of the intermediate substance $I$, which may be cytochrome, "ferment," or any other respiratory catalyst:

$$O_2 + I \rightarrow I\,O_2$$

$$I\,O_2 + S \rightarrow I + S\,O_2$$

when $S$, the substrate, is present in excess and if we are concerned only with the oxidation and reduction of $I$, the reaction may be simply represented by the scheme:

$$I + O_2 \rightleftarrows I\,O_2$$

Assuming further that $A/A_0$ is a measure of the relative concentrations of $I$ and $I\,O_2$, their expression relating $A/A_0$ and $P$ may be stated in the following manner:

$$\frac{n}{(1 - n)P} = K$$

in which $n = A/A_0$, or,

$$\frac{A}{A_0} = \frac{KP}{1 + KP} \quad (2)$$

Transposing,

$$A = \frac{A_0 KP}{1 + KP}$$

Letting $1/KA_0 = K_1$ and $1/A_0 = K_2$, we have:

$$A = \frac{P}{K_1 + K_2 P}$$

which is similar to our empirical equation, and in which $1/K_2$ should be equal to $A_0$ and $K_2/K_1$ should be equal to $K$.

It is noted that our empirical equation also resembles the one developed by Langmuir (1918) for the adsorption of gases on solid surface: viz.,

$$q = \frac{abc}{1 + bc}$$

in which $q$ = amount of gas adsorbed per gram solid, $c$ the equilibrium concentration and $a$ and $b$ are constants. Indeed, if we make the simple assumption that the rate of $O_2$-consumption ($A$), is proportional to the amount of $O_2$ adsorbed ($q$) on the respiring surface, the equation for adsorption may be considered applicable to this phase of respiration and a mechanism may be postulated for the mode of combination of $O_2$ with the respiring material on the basis of spatial arrangements of the molecules on solid surface as pictured by Langmuir (1918). This line of thought has to a certain extent been adopted by Freundlich and Fischer (1925), by Rideal and Wright (1925, 1926, *a*, *b*), by Quastel (1926), and by Shoup (1929–30). But since the question of the mode of combination of $O_2$ with the respiring material is far from being settled at present, it is perhaps wise for us to stop at the statement that the present equation expressing the rate of $O_2$-consumption in tissues and lower organisms as a function of $O_2$ tension is purely empirical, and until many more quantitative experiments are available pertaining to this question it is best not to attach too much significance to the constants of the equation. The mathematical identity of our equation with those derived for adsorption, dissociation, and reversible oxidation and reduction need not be taken as conclusive evidence supporting their physical identity. Among the recent accounts of the mechanism of oxidative catalysis in respiration may be mentioned those of Dixon (1930), Michaelis (1929), Elliott (1930), and Meyerhof (1924) and the literature cited in these papers.

It is noted that our treatment of the respiration data does not take into consideration the morphological aspect of the respiratory mechanisms such as those existing in fish and insects. It is assumed here that as long as the data secured with these organisms can be treated with the simple relation, as expressed by equation (1) or (2), it is immaterial to know, for our purposes, through what morphological channel the $O_2$ was made available. There are undoubtedly cases where morphological modifications of the respiratory mechanism occur as the $O_2$ tension is varied, and other factors as well as simple diffusion may come into play. In such cases, the data are not suitable for the kind of analysis used here. For this reason, it is desirable that future work dealing with the kinetics of cell-oxidation be performed on unicellular organisms. For a discussion of the morphological aspect of the respiratory mechanisms in the lower animals see Rogers (1927). A comprehensive morphological and ecological treatment of respiration in the fishes as related to $O_2$ tension is given by Powers *et al.* (1932). A similar account for insects is given by Wigglesworth (1931).

## VI

We shall now treat equations (1) and (2) once more and attempt to analyze the behavior of the constants.

$$\frac{A}{A_0} = \frac{KP}{1 + KP} \quad \text{or} \quad A = \frac{A_0KP}{1 + KP}$$

Setting $1/A_0K = K_1$ and $A_0 = 1/K_2$, we have:

$$A = \frac{P}{K_1 + K_2P}$$

or

$$\frac{P}{A} = K_1 + K_2P$$

It is to be noted that when $P/A$ is plotted against $P$, $K_1$, the intercept, corresponds to $1/A_0K$ and the slope $K_2$ is $1/A_0$. Since by experiment we know that $\frac{d\ln A_0}{dT} = \frac{\mu}{RT^2}$ (the Arrhenius equation) in which $\mu$ is a constant termed the "temperature characteristic" by Crozier (1924–25), $K_2$ should vary with temperature in such a way that when $P/A$ is plotted against $P$, an increase in temperature will cause a decrease in the slope ($K_2$) of the line in the manner described by $\frac{d\ln A_0}{dT} = \frac{\mu}{RT^2}$. $K_1$, the intercept, contains the terms $1/A_0$ and $1/K$. While we know from above that $K_1$ is partially decreased by an increase in temperature because of the dependence of $A_0$ on temperature, the change of $K_1$ with temperature cannot be predicted without knowing the relation of the latter to $K$. The only experimental evidence on the change of $K$ with temperature is that furnished by Warburg and Kubowitz (1929) with *Micrococcus candicans*. Their data for $\frac{n}{(1-n)P_{O_2}}$, (or $K$), ascertained for *Micrococcus* at 1°, 5°, and 10° are plotted on Fig. 3, together with the data for respiration at these temperatures. It is evident that $K$ has a negative temperature coefficient ($\Delta H = -24{,}000$ cal.) while $A_0$ has a positive temperature coefficient of $\mu = 30{,}500$ cal. If we use the expression $\frac{d\ln K}{dT} = \frac{\Delta H}{RT^2}$ (which is van't Hoff's reaction isochore) as an approximation in describing the relation existing between $K$ and $T$, we see that the decrease in $K_1$ caused by $\frac{d\ln A_0}{dT}$ will be more or less compensated by $\frac{-d\ln K}{dT}$, and $K_1$

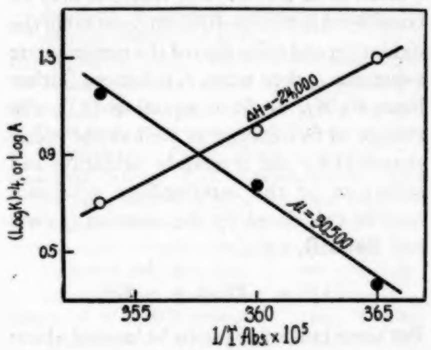


Fig. 3. Log K $\left(\text{or Log } \frac{n}{(1-n)P_{O_2}}\right)$ and Log $A$ (or Log $Q_{O_2}$) are Plotted Against $1/T$

Data taken from Warburg and Kubowitz (1929) for *Micrococcus candicans*. The open circles are for the values of $K$, and the solid ones are for $A$. $A$, $K$, $P_{O_2}$ and $T$ are rate of respiration, equilibrium constant, $O_2$ tension in atmospheres, and the absolute temperature, respectively. $n = \frac{A}{A_0}$ in which $A_0$ is the rate of respiration in air (maximum rate). Since the points are taken at temperatures which are quite far apart, the slopes of the lines ($\mu$) and ($\Delta H$) should be considered only as approximations. The possible occurrence of a "break" in both curves at 5° ($360 \times 10^5$ $1/T$) must be recognized.

should be more or less independent of temperature according to the relative magnitudes of $\mu$ and $\Delta H$. While no comprehensive data whatsoever have been obtained which may throw light in this direction, it is interesting to compare the constants obtained with fertilized *Arbacia* eggs by Amberson and Tang and Gerard

(nos. 14 and 17 in Table 1). The data of the former were obtained at about 18° and those of the latter at 25°. It is evident from Table 1 that while the values of $K_1$ are almost the same in both cases (6.0 and 6.5) $K_2$ decreases from 0.95 at 18° to 0.55 at 25°. However, until much more comparable data are obtained, these calculations must be considered only as speculations.

In cases where the assumptions used for the derivation of equation (2) may be considered to be correct, and where K may be considered as the equilibrium constant of the oxidation and reduction of the intermediate substance $I$, then when K is known (either from $K_2/K_1$, or from equation (2)), the change in free energy as well as the redox potential for the reversible oxidation and reduction of the intermediate substance may be calculated by the relation (Lewis and Randall, 1923):

$$\Delta F^0 = -RT\ln K = -nFE^0$$

But since much has yet to be learned about K, such calculations must be postponed.

Whatever the theoretical interpretations of the constants may be, the formal relationships existing among $A$, $P$, and $T$ may be described by the three equations:

(1) the equation of Warburg and others

$$A = \frac{P}{K_1 + K_2P}$$

or

$$A = \frac{A_0KP}{1 + KP};$$

(2) the Arrhenius equation, as adapted to biological processes by Crozier:

$$\frac{d\ln(1/K_2)}{dT} = \frac{d\ln A_0}{dT} = \frac{\mu}{RT^2};$$

(3) the reaction isochore of van't Hoff:

$$\frac{d\ln(K_2/K_1)}{dT} = \frac{d\ln K}{dT} = \frac{\Delta H}{RT^2}$$

When the constants $K_1$, $K_2$, $\mu$ and $\Delta H$ are known, $A$ may be evaluated for any combination of $T$ and $P$ at which the three relations hold. Thus $A$ is completely and quantitatively defined with regard to $T$ and $P$, which in the simpler cases are the only variables of any consequence. Such a method of description differs from the "principle of limiting factors" of Blackman (1905) in that the latter is a qualitative statement of the change of a physiological process when one factor is varied at a time while the others are maintained at a maximum.

## VII

To recapitulate: While the hyperbolic type of equation holds for the great majority of the data examined in this account, it is also true that data exist where the equation fails to hold strictly without undue modifications, as in the cases where the relation between oxygen tension and the rate of oxygen consumption is apparently linear. In such cases the relationship may be considered as being complicated by the presence of diffusion gradients in the tissues or in the media, or by the rate of circulation of the blood stream, etc., and it may merely be expressed empirically by $A = kP$. It is only in the last few years that attempts have been made to elucidate these factors, and mathematical expressions have been arrived at to approximate their relative rôles in respiration (*cf.* Shibata and Tamiya, 1930; Gerard, 1931; and Roughton, 1932). As yet none of these suffices to account for the available data in a quantitative way. At the same time the technic of measuring respiration has been greatly refined with the purpose of eliminating errors due to insufficient supply of $O_2$ to the respiring materials. In view of these recent developments, future work relating the rate of $O_2$-consumption and $O_2$ tension should be directed at sim-

plicity, and the materials and methods should be so selected that the number of uncontrollable and unknown variables be at a minimum. Where such rigorous requirements cannot be met, as in ecological studies, it is hoped that at least the results may be expressed in a readily intelligible and if possible, uniform manner, so that the constants, when ascertained under comparable conditions, may provide a basis for comparing the habits of different organisms.

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# THE PROBLEM OF ACQUIRED PHYSIOLOGICAL IMMUNITY IN PLANTS (*Continued*)

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## VII. ACQUIRED IMMUNITY IN PARASITISM

WE NOW come to the most interesting and practically most important aspect of the problem of acquired immunity, namely the acquired immunity displayed in nature. Abundant opportunities are offered in nature for sensitization. First and foremost one immediately recalls parasitism with its wealth of likelihood of the display of immunological phenomena. But no less interesting and instructive are the possible immunological phenomena attending the sensitization due to symbiosis of various sorts. We shall accordingly devote the present and following sections to a consideration of acquired immunity in parasitism and symbiosis respectively.

In order that the proof of acquired immunity be complete in these cases of natural sensitization it is necessary not only that immunological phenomena be observed *in vivo* (recovery from disease, resistance to superinfection, peculiarities of behavior of the foreign body within the host tissues, etc.), but also that it be demonstrated that such reactions are due to antibodies. This dual proof, the necessity for which has been well pointed out by Silberschmidt (161), has been resorted to in very few of the experiments and observations in this field, yet taken as a whole the evidence thus far available, as we shall see, renders very sound the hypothesis that reactions of the zoöimmunitary type do occur in plants and may play an important rôle in the preservation of plants in nature.

### *A. Immunity to reinfection*

The most obvious manifestation of acquired immunity in parasitism is the complete or partial immunity of a host to reinfection after recovery from an earlier attack of a disease. Numerous observations of such immunity to reinfection have been made in the past twenty years and while all may not be due to acquired immunity in the zoöimmunitary sense, the evidence is certainly strongly suggestive of such in some cases at least.

First in point of time should be mentioned an observation of Smith and his colleagues Townsend and Brown in 1908 and 1909 (Smith, 1911: 162). These investigators obtained some evidence to show that after Paris daisies had been several times inoculated with *Bacterium tumefaciens* with the production of tumors, subsequent inoculations with cultures of the same organism were without effect. Later experimentation showed that this assumed increased resistance, however, was due, at least in part, to a loss of virulence on the part of the parasite. Smith likewise thought for a time that he had achieved resistance to the olive tubercle organism in plants which had been freely and repeatedly inoculated, but that too may have been due at least in part to loss of virulence of the parasite. In a second publication the same year (163) these workers

reported more extensive experiments with *Bacterium tumefaciens* on daisy in which the error due to loss of virulence was effectively eliminated in most of the experiments. Successive vegetative propagation and inoculation of Queen Alexandra daisy showed a decided refractoriness to infection after the third set of inoculations and vegetative propagation. This resistance was manifested in the extremely slow growth and small size of the galls. The virulence of the strains of bacteria was controlled in each case by the infection of fresh (never-infected) daisy or beet with the production of large, typical galls in a brief period. This work was continued by Brown in 1923 (27) when she reported that attempts to build up an acquired resistance of daisy and rose to the same parasite by repeated infections and vegetative propagation from such infected stock failed to show any permanent resistance, although a temporary acquired resistance was twice noted.

Arnaudi two years later (4) noted that the presence of a crown gall on geranium inhibited reinfection a few centimeters from the preëxisting tumor, and in 1928 the same worker published a further account of work with *Bacterium tumefaciens* (7) in which he found a somewhat greater susceptibility in geraniums which had never been infected than in plants with recent or old crown galls. Unfortunately Arnaudi's results were based on the reactions of only ten plants including controls, and since his difference between controls and experimental plants is not great one regrets that an accurate quantitative increase in resistance could not be accurately determined. Moreover, Nobécourt's experiments of the same type (136) failed to show any protective effect of preëxisting crown galls in *Pelargonium* and *Euphorbia*, a failure which Nobécourt believed might have been due to the distance between gall and inoculation. Finally a few additional experiments with this same organism were performed by Riker in 1926 (152). Riker inoculated with *Bacterium tumefaciens* plants both with and without galls but he failed to detect any difference in reactivity between the two types of plants. The possibility that antibodies had been produced but not circulated was rendered doubtful by his failure to find bacterial agglutinins, precipitins, or lysins in the gall extracts.

The only other experiments dealing with the effect of infection with bacteria on subsequent reinfection by the same bacterial species are those of Němec in 1929 (133) in which he observed an acquired immunity to *Bacillus pyocyaneus* (destructive by means of its toxins) in green varieties of cabbage, but not in blue varieties, and in *Crassula lactea*. Such acquired immunity was found to be somewhat restricted to the point of inoculation, however, and not freely circulated, although the inoculation of one side of the *Crassula* leaf offered a partial protection against the deleterious effects of subsequent inoculation of the other side of the same leaf.

Beside these experiments with increased immunity to reinfection by bacteria, observations of similar phenomena have been made regarding other types of plant disease. Thus in the case of fungus diseases of plants we have several such records. In 1911 and 1914 (170, 171) Tischler made some very interesting observations on the rust disease of *Euphorbia cyparissias* caused by *Uromyces pisi*. He found that it was possible to induce rapid and complete recovery from the rust attack by placing the affected plants in a saturated atmosphere at relatively high temperatures. Once such recovery took place the shoots were then no longer susceptible. Viable mycelium in the older portions of the plant was unable to infect the new growth and was even killed back. A similar effect was also accomplished by eliminating the win-

ter rest period of the host, in which case the mycelium remained viable in the vacuolated cells of the tips of the branches but was unable to penetrate the meristematic tissues of the vegetative point. Tischler interpreted these results in terms of nutritional and osmotic relationships between host and parasite, but it seems equally probable that his results are susceptible to an explanation in terms of an acquired physiological immunity. Further experiments *in vitro* would be necessary in order definitely to establish the truth of either of these hypotheses.

Montemartini in 1918 (121) observed that in that year the European oak suffered less than in preceding years from the mildew (*Oidium*). This decrease in the disease appeared to be due not to environmental conditions during that year but rather either to a diminished virulence of the fungus or to an acquired immunity of the host. Montemartini continued his observations for the following twelve years and by 1930 (122) was of the opinion that the disease was gradually becoming much less severe in general than in earlier years. Various hypotheses had been advanced to account for this decrease in the severity of the mildew, none of which had been supported by experimental proof. Montemartini accordingly performed a series of inoculation experiments in order to detect possible acquirement of immunity in parasitized trees. Twelve young oaks were used, of which six were protected from the disease in 1929 while the other six were permitted to become heavily infected. The following year all twelve trees were exposed to the disease. The six previously protected plants all showed heavy infection while the six previously exposed plants with one exception remained free from the disease.

A very similar type of observation was made in 1930 by Gravatt and Gill (70), who believed that the coppice growth from chestnuts killed by *Endothia parasitica* was annually becoming larger and stronger before succumbing to the bark disease. Just as in human disease so in plant disease one observes that all epidemics in time tend to become less and less severe. This waxing and waning of specific diseases is doubtless the resultant of many interwoven factors of which virulence of the parasite and susceptibility of the host are the most important. We have before us evidence that two such epidemics are now on the wane. In one case we also have evidence that this diminution in severity accompanies an acquirement of immunity in individual plants. Regarding the chestnut bark disease we have no experimental evidence to guide us, yet the sequence of events in the case of the oak mildew might afford a valuable clew to the understanding of the *Endothia* disease. Other epidemics are now threatening us: the devastating Dutch elm disease is at our door, the very destructive willow scab disease is sweeping down from the north and fast invading the northeastern states, the same is true of the beech canker disease and others. Sooner or later we must learn how successfully to combat these epidemics, and our experiences with the oak *Oidium* and the *Endothia* canker may have much to contribute to an understanding of the host-parasite relationship which must serve as the groundwork for our control of such epiphytotics.

We are indebted to Whetzel for a third observation of this same order (186), an observation which may best be related in Whetzel's own words:

> During my senior year in Wabash College I made some studies of the *Gymnosporangium macropus* which occurs very abundantly on cedars and apple trees about Crawfordsville, Indiana. I observed that certain cedar trees were very badly infected, being loaded with galls, large and small, on all their

twigs and branches. Other trees standing near were almost or quite free from any infection. A couple of years later I returned to Crawfordsville for a visit and went out again to see the cedar trees from which I had, during my senior year, gotten such large quantities of galls. To my astonishment they were practically free from infection, while others nearby that had borne no galls before were now badly covered with them. What the explanation of this phenomenon is I do not know. It occurred to me, however, that a serious infection of the trees one season might have rendered them more or less immune for a time. That the infection was on different trees in these two years is certain, as I was very familiar with the different trees with which I had worked.

Another observation of the same type concerns the *Hemileia* rust of coffee, as reported by Dowson in 1921 (56a). Dowson found that in East Africa the first attack of the rust disease is much more severe than subsequent attacks, from the standpoints of the number of rust pustules per leaf and of the number of leaves infected. This fact was interpreted by Dowson as indicative of an acquired immunity, since observations of adjacent plantations suffering from first and subsequent attacks of the disease showed that the virulence of the parasite remained constant.

Beside these more or less clear-cut cases of acquired immunity to fungus infection may be mentioned in passing the experiments of Doussain in 1925 (56). This worker found that if onions were infected with various molds, then crushed and mixed to form a paste and reinfected, the previously infected onions molded more quickly than those which had been not previously molded. This would seem to indicate a situation exactly contrary to that reported for oak *Oidium* and *Euphorbia* rust, i.e. a greater *susceptibility* following infection, but unfortunately Doussain's data are not sufficiently complete for one to draw suitable conclusions from his experiments. It may have been that the increased "susceptibility" shown was merely due to the presence of an abundance of mold spores from the earlier infection.

We have considered acquired immunity to reinfection in diseases caused by bacteria and by fungi. There yet remain two other important types of plant disease which have also been investigated in this connection, namely the diseases due to phanerogamic parasites and those due to viruses. Our evidence regarding the former is restricted to the findings of Heinricher regarding mistletoe infection of pear (71, 72). This investigator distinguished three relationships between pear and *Viscum album*, namely "true immunity," "true susceptibility," and "false immunity." The first two terms are self-explanatory. In the case of "false immunity" the pear shows an immunity to infection but not to the toxins of the parasite. Some of the "susceptible" plants were able to throw off the attack of the parasite and recover. Second and third infections were sometimes possible but lead only to very mild symptoms in the host as compared with the much more violent reactions of the host to the first attack. In many cases (97 per cent) reinfection was not possible after recovery from an attack of the parasite. This ability more successfully to withstand subsequent attacks was interpreted by Heinricher as being due to the acquirement by the host of "antitoxins" capable of neutralizing the toxins of the parasite, although he offered no further experimental proof of this hypothesis.

Finally, with regard to the virus diseases several investigators have contributed to a conception that plants recovering from such diseases do so through the agency of an acquired immunity. It is indisputable that plants suffering from virus diseases may recover in the sense that disease symptoms are no longer present; this has been amply demonstrated in the following cases: tobacco mosaic (Beijerinck, 1898: 11); *Abutilon* mosaic (Baur, 1906: Ber. Deut. Bot. Ges. 24: 416–428); tomato mosaic

(Brierley, 1915: 26); corn mosaic (Brandes, 1920: Jour. Agr. Res. 19: 517–522); sugar beet mosaic (Robbins, 1921: Phytopath. 11: 349–365); sugar cane mosaic (Kunkel, 1924: Hawaiian Sug. Cane Plant. Ass. Bull., Bot. Ser., 3: 115–167); sugar cane corn streak (Storey, 1926: So. African Jour. Sci. 23: 305–306); tomato mosaic (Verwoerd, 1929: 177); tobacco cucumber mosaic (Johnson, 1930: Kent. Agr. Exp. Sta. Bull. 306: 285–415); sugar cane mosaic (East, 1930/31: 60, 61, 62); cucumber mosaic (Porter, 1931: Iowa State Coll. Jour. Sci. 6: 95–129); tobacco virus diseases (Thung, 1931: 169a); and tobacco ringspot (Price, 1932: 142a).

But we must further indicate what is meant by "recovery." The problem in other words concerns the question whether plants no longer displaying symptoms still harbor the virus or not. If the virus is still present we may consider the disease as present but "masked" as in human pathology in the case of the typhoid "carrier." If the virus is no longer present we may consider the plant as showing *true recovery*. The literature as mentioned above contains examples of both masking and true recovery. From the standpoint of immunology both are of interest, since in both cases the freedom from symptoms may be attributable to an acquired immunity. In order to establish the proof of acquired immunity in such cases the experiments must include the following steps:

A. Are the "recovered" plants still infectious (masking) or not (true recovery)?

B. Whichever is the case, are the plants now immune to the virus originally used in infection (proof of acquired immunity)?

C. Are the plants still susceptible to other "species" of virus (specificity of the acquired reaction)?

All of these steps are desirable in proofs of the type before us. Let us see how thoroughly the experimental data serve to elucidate our theme.

1. With regard to the references cited above, in many cases cited the observations have not gone beyond the point of determining that plants have *recovered* from virus diseases. This applies to the works of Beijerinck, Brandes, Kunkel, Storey, and East.

2. Others have carried the matter one step farther; experiments have been performed to show whether the "recovery" was *masking* (true in the experiments of Robbins, Johnson, and Porter) or *true recovery* (true of the experiments of Brierley and Verwoerd). These workers, however, did not go on to the crucial experiments directly ahead, namely experiments to determine whether the plants were now immune.

3. Baur's plants showed *true recovery*, and reinfection with the same strain of virus originally employed showed them to be now immune. The specificity of this immunity was not investigated, however, and furthermore Baur's experiments are not considered very convincing. On the other hand, Price (Contr. Boyce Thomps. Inst. 4: 359–403. 1932) who was dealing with a case of *masking* found that the plants were now immune to reinfection with the same virus as originally employed, that the masked virus and the immunity continued through three vegetative generations with undiminished potency, and that immune substances were not transmitted from stock to scion through the graft union. Price did not test the reaction of his recovered plants toward other types of virus with a view to ascertaining the specificity of the immunity. Thung (Handel 6de Nederl. Ind. Natuurwetensch. Congr.: 450–463. 1932) who also observed complete recovery as well as masking found that plants diseased with one type of virus

were subsequently immune to a second "species" of virus. Birkeland (Phytopath. 23: 5. 1933) found no evidence of acquired immunity in tobacco mosaic and spot necrosis, but he was dealing with plants which showed neither recovery nor masking.

The evidence from the virus diseases is thus highly favorable to the theory of immunity acquirement in plants, and this is still further strengthened by Salaman's data on vaccination with viruses (Nature 131: 468–470. 1933) which show that infection of tobacco plants with an attenuate form of potato virus renders them specifically immune to later infection with a virulent form of the same virus. The facts as set forth are these: plants suffering from virus diseases may recover from their disease symptoms; in such "recovery" the virus may or may not still be present in the plants; whether or not virus is still present, such plants wherever tested have been found to be immune to further attacks by the same virus; this immunity has been shown to persist through three vegetative generations but not to be due to a freely circulated humoral substance: its persistence may well be due to the constant stimulation by the masked virus which is present. These data show that acquirement of immunity to virus diseases is a fact, and the last two points further indicate that the immunity thus acquired is closely bound up in the living cell, not freely diffusible, a point which will be stressed subsequently but one which in no way detracts from the thesis before us.

Accordingly we have before us a fairly significant body of data regarding increased resistance to reinfection after a first attack of a disease. These data afford answers, progressive if not complete, to certain important questions which confront us after a consideration of this subject. The first of these questions, namely whether or not a plant may show an increased resistance after one parasitic attack, would appear to have been answered in the affirmative. Several investigators have demonstrated such an acquired immunity after bacterial attack, fungus infection, and parasitism by a phanerogamous parasite. Although the number of investigations in this field is as yet limited it is significant that practically all are mutually confirmatory, and lead to the conclusion that plants may acquire an increased resistance after a primary attack of a specific disease.

Yet one must qualify these results and view them with a certain amount of caution. For in the first place few if any of these experiments considered alone are satisfactory proofs because of the failure to consider certain sources of error inherent in this type of experimentation. Thus in the first place all the investigations appear to indicate that the immunity to reinfection acquired by plants as the result of a first attack of a disease may be relatively weak, temporary, or localized. Accordingly particular emphasis must be laid on the detection of small but significant differences between control plants and experimental plants. This of course implies that both controls and experimental plants must be employed in sufficient quantity to make possible statistical studies. Experiments involving a few plants are of value in orientation, but the actual tests of acquired immunity should involve scores or hundreds of plants. Furthermore, since resistance and immunity are conditioned by a number of genetic and environmental factors, particular emphasis must be laid on the elimination of such normal variables. For example, that a plant is resistant to reinfection may mean that it has acquired immunity as the result of a first infection, but it may likewise mean that its greater

maturity at the time of the second attempt has increased its resistance, or that the environmental conditions surrounding the second attempt were not as favorable to infection as those attending the primary infection. One must therefore use a complete and extensive system of controls in order to eliminate errors due to such causes. Finally, in such experiments as this, one must conform to a complete schedule of proof just as in the proof of the causation of a parasitic disease one conforms to the postulates of Koch. In the present instance such a schedule should include:

1. A satisfactory primary infection shown to be due to the parasite in question.
2. Recovery from the primary infection.
3. Infection experiments to determine the susceptibility of such recovered plants at varying lengths of time after recovery and employing strains of the same pathogen of virulence equal to that of the strain causing the primary infection.
4. An adequate system of controls demonstrating that plants in every respect the same as the experimental plants except that they lack the primary infection are susceptible to the same parasite in a greater degree than the test plants at the time of the second infection.
5. In cases where there is likelihood of any doubt upon the question, the determination of whether the plants showing recovery have truly recovered or are merely harboring the parasite or infectious principle under such conditions as to produce no visible pathological symptoms. This particularly applies to a study of the virus diseases.
6. Determination of the degree of acquired immunity by the employment of some accurate mathematical measure; also determination of the duration, extension, and specificity of the acquired immunity.
7. A very valuable supplement to such experiments would be the comparison of the experimental results with observations of the disease in the field, as has been done in one case described above.

That immunity to reinfection may be acquired by plants appears evident from the experiments reported above, although more extensive and thorough experiments are needed to establish this point. That such acquired immunity may play an important part in the natural control of diseases in the field is likewise evident both from the theoretical relation of the experiments to plant pathology and from actual observations in the field such as those of Montemartini concerning oak mildew. As yet we have no adequate control of this phenomenon, but with the knowledge gained from such experiments as those described above we are equipped to carry into the field of practical phytopathology a new weapon of defense, biological prophylaxis. Before considering the part this may play in practice, however, it is first wise to discuss the experiments which have been performed dealing with the vaccination of plants, which will accordingly be the subject of the next few pages.

### *B. The vaccination of plants*

We employ the borrowed term "vaccination" to include various methods of biological plant therapy. Vaccination in the medical sense implies the treatment of a subject with an attenuated strain of a parasite, the mild parasitism resulting conferring upon the subject an immunity to subsequent attacks of virulent strains of the same or related organisms. In the sense of the present paper, however, beside

this procedure others are included in the term vaccination. Thus the subject may be immunized subjectively (actively) by the introduction of an attenuated strain of a parasite, or objectively (passively) by the introduction of antibodies of either plant or animal origin. All of these methods have been attempted with plants, and will accordingly be successively considered.

First among the experiments in the *vaccination of plants with attenuated parasitic strains*, and indeed first in the field of acquired plant immunity, must be mentioned the findings of Ray (147) and Beauverie (10) in 1901. The contributions of these two investigators were so similar in many respects that they may be considered together. Beauverie in 1899 (9) reported that by the use of certain environmental variables (heat, cold, humidity, poor nutrition) he was able to vary the virulence of a sterile form of *Botrytis cinerea* ("toile") at will. In this way he was able to obtain strains of the "toile" which were sufficiently attenuated as to cause only a mild, temporary attack of the grey mold. Ray also accomplished the same end in 1901 (146) by the cultivation of *Botrytis cinerea*, a bacterium parasitizing legumes, and other parasites at high and low temperatures. Both workers then vaccinated susceptible plants with the attenuated strains thus obtained and observed the effect of this vaccination upon subsequent susceptibility. Beauverie immunized by planting *Begonia* in soil saturated with the attenuated *Botrytis*. The plants were not sensibly affected by such treatment. Subsequent transferal of such immunized plants to environments rich in virulent *B. cinerea* revealed that the plants resisted the parasite perfectly although non-vaccinated controls soon died from the effects of the virulent strain. Ray's experiments in vaccination are reported in much more detail than those of Beauverie and appear to be thorough and sound. Rigid attention was paid to such factors as sterility of soil and of seeds, purity of parasitic cultures, etc. The preventive inoculation was performed by injection of the plants at many points employing several preventive inoculations either with strains of equal attenuation or preferably with a series of strains of increasing virulence. The preventive inoculations resulted in slight infections from which the plants soon recovered, while the subsequent inoculations with virulent strains of parasites were wholly without effect.

For a long time these results of Ray and Beauverie remained unconfirmed, but during the past ten years several investigators have again taken up the same question. Brown's work with *Bacterium tumefaciens* on daisy and rose (27) has already been mentioned in another connection. Beside her findings regarding the resistance of galled plants to reinfection Miss Brown reported a few attempts in vaccination of these plants using dead bacteria as a vaccine. She found that vaccination of a point in the stem a short distance from a second inoculation one day later with virulent bacteria exerted no protective action against the second inoculation, but if the protective and virulent inoculations were made at the same point, only 20 per cent of the inoculations yielded galls as compared with 100 per cent in the first case. Miss Brown was not dogmatic in interpreting these experiments in the light of an antigen-antibody effect and wisely, because a double injection in the same point of a stem might well lead to errors due to the presence of inhibitory substances in the first inoculum. However, her failure to obtain protection at a short distance from the first inoculation is hardly conclusive evidence of the lack of biological protection because of the very short time

for antibody production and transportation (1 day) and her results in double inoculation of the same point are of interest in that they confirm the results of the investigations of Ray and Beauverie. Gheorghiu (67) has very recently performed an extensive set of experiments in vaccinating *Pelargonium* against crown gall using heated cultures of *Bact. tumefaciens* as vaccine. He obtained a strong specific protection and cure lasting three months.

Arnaudi has performed some experiments in this field during recent years working with a bacterium causing potato decay, *Bacillus mesentericus* (4, 6, 47). He attenuated the pathogen by ageing at 37°C. and gradually adding lactic acid to broth cultures of the organism. This vaccine was applied to potato slices and subsequent inoculation of the virulent strain of the bacillus caused less severe and less rapid decay in the vaccinated potato slices as compared with the controls. Although his experimental tubers do not appear to be very numerous and although his results were not always very striking, yet his positive cases of decreased susceptibility after vaccination are sufficiently frequent to preclude the possibility of their being fortuitous, and Arnaudi's findings accordingly extend and substantiate those of the earlier workers.

The experiments of Beauverie with attenuated *Botrytis cinerea* have very recently been confirmed by Carbone and Kalajev (49a). These investigators also found that vaccination with the "toile" conferred an immunity on the host, although different techniques of vaccination yielded somewhat different results. The explanation of this discrepancy was found by Carbone and Kalajev to be due to a strict dependence of the acquired immunity upon a vital condition of the host. Injured or intoxicated hosts were less able to defeat the aggressive pathogen, and accordingly the vital character of acquired immunity in such vaccinated plants seems evident.

Vasudeva in 1930 (175) reported experiments in the double inoculation of apple fruits using successive inoculations of different organisms as well as inoculations with mixed cultures. In general he found that the presence of certain saprophytic fungi in association with certain parasites (as *Botrytis allii* in combination with *Monilia fructigena*) reduced the virulence of the parasitic attack. Vasudeva concerned himself with the question of whether the presence of the saprophyte resulted in an immunizing action upon the apple directed toward the parasitic fungus, and came to the conclusion that the decreased susceptibility shown could better be explained on the assumption of staling phenomena than by an immunological hypothesis. However, it does not appear from this work that there is sufficient ground for assuming that it either favors or argues against an immunological hypothesis. It is certainly difficult to conceive of the protective action of a "vaccine" of this sort administered at the same time as the virulent organism, and furthermore one would hardly expect that in any case vaccination with one species of fungus would cause an immunization against another very distantly related fungus. We have no analogy to such a situation in zoötherapy either in theory or in practice. Accordingly Vasudeva's results do not appear to afford any serious objection to the positive findings of other workers in this field.

In an unpublished lecture at Harvard University Dr. M. L. Rane mentioned work which he and others had done at the University of Chicago in the vaccination of plants (145). Taking advantage of the fact that "rough" bacterial strains are in general less virulent than "smooth" strains Rane and his associates performed

a few vaccination experiments but without success. Unfortunately the details of these experiments are not available as yet.

We thus see that a fairly comprehensive start toward a study of the vaccination of plants with attenuated forms of pathogens has yielded results which are very suggestive. Ray, Beauverie, Arnaudi, Brown and Carbone and Kalajev have all found that such vaccination resulted in a moderate increase in immunity. The available evidence of Rane's is as yet too incomplete to serve as a creditable objection to the findings of others, and moreover even though extensive negative results had been obtained, these would not serve to disprove the thesis, since in the problem at hand we have one in which, because of the exacting conditions of experimentation necessary to demonstrate positive findings positive results, if carefully controlled, are significant, while negative results are not necessarily so. Just as has been found in the display of acquired immunity following recovery and reinfection, so here in vaccination with attenuated pathogens we find that the degree of immunity conferred is not strong in all cases nor is it easily distributed through the plant, both of which again emphasize the need for complete and carefully controlled experiments in this field.

*Vaccination with extracts of parasites* is a second type of vaccination which has been attempted by a number of workers in the field of acquired plant immunity. Ray again inaugurated this type of investigation when he found in 1901 (147) that the water-soluble components of the alcoholic precipitate of virulent parasitic cultures if sprinkled upon the soil surrounding the roots of a susceptible plant confer upon that plant the same immune properties as if the plant in question had been subjected to infection by an attenuated strain of the parasite. Ray was not specific with regard to which particular host-parasite combinations he employed in this connection, but stated it as a general principle derived from observations of a number of host-parasite couples.

In 1925 Miss Zoja performed a similar experiment (192). She germinated seeds of *Triticum vulgare* in aqueous extracts of *Helminthosporium sativum* and of *Triticum* plants affected with this fungus and observed that after a few days the plants showed temporary signs of distress. Subsequent treatment with fresh *Helminthosporium* extract, however, resulted in no symptoms of disturbance, and when the plants were then exposed to potent *Helminthosporium sativum* they showed a complete immunity which lasted for at least one month. The active immune principle was found to be stable at temperatures below 50°–55°C. although it was destroyed by boiling. It appears from Miss Zoja's experiments, which were apparently carefully controlled and thoroughly executed, that she was dealing with a true case of acquired immunity induced by the antigenic functioning of the toxins or proteins present in the extracts.

During the same year Hursch (75) published an interesting account of experiments of this same general type. He cultivated parasitic fungi in liquid solution and then noted that such filtered solutions if administered to the host species produced in the latter symptoms of wilting resembling those of the parasitized hosts (as by *Fusarium*). However, plants so treated (e.g. cabbage and cauliflower) soon recovered when placed in fresh water, and if such plants were again placed in the same fungus filtrate they remained perfectly healthy, although untreated controls rapidly wilted in the fungus filtrates. There was a certain amount of individual variation among the plants in regard to their reaction to fungus filtrates which is

not to be remarked at since there is also much individual variation toward disease in the field. The nature of the toxic and antigenic components of the extracts was investigated and it was found that substances with various properties were responsible, although in general the severest symptoms were produced by those substances which were non-precipitable by alcohol, dialyzable, adsorbed by animal charcoal, and thermostable. They hence appear to be non-protein although apparently organic in nature and accordingly lend credence to the view that non-protein organic substances may in some cases be antigenic. On the whole Hursch's results appear to be significant, although details regarding his experimental procedures would be highly welcome.

The work of Sieden and Trieschmann reported in the following year (158) is of especial interest because it combines a theoretical study of acquired immunity resulting from treatment with parasite extracts with a practical application of such a procedure. These investigators worked with the serious potato wart disease caused by *Synchytrium endobioticum*. The first experiment in 1925 consisted in inoculating seed tubers with extract of overwintered warts by the use of a syringe. Such vaccinated potatoes, however, failed to show greater resistance than non-vaccinated controls. In 1926, on the other hand, the technique was varied in that the wart extract was introduced into boreholes in the tubers which were then sealed again. The results of this second experiment were highly satisfying. In the fall the vaccinated potatoes had been attacked only slightly by the disease in a thoroughly infected field, while the controls showed numerous warts. Sieden and Trieschmann grant that as yet almost nothing is known regarding the comparative values of various techniques, but as their second experiment shows even these first tentative attempts have yielded results of significance in the economic struggle against the wart disease.

Arnaudi in 1928 (7, 47) performed some comparable experiments with a disease of *Pisum sativum* caused by *Blepharospora cambivora*. Pea seeds were germinated in (a) water, (b) extract of *Blepharospora*, (c) the same, but which had been heated to 60°, and (d) to 100°. The seeds in all cases but (b) continued to grow. When then inoculated with *Blepharospora* the vaccinated plants (c and d) were able to resist the attack and survive two weeks longer than the non-vaccinated controls, although eventually all were killed. The specificity of the vaccination was investigated in an exploratory way by vaccinating two lots of seedlings with *Aspergillus oryzae* and *Blepharospora cambivora* respectively. The seedlings were then all subjected to infection by *Blepharospora* with the result that the vaccination with *Aspergillus* afforded no protection against *Blepharospora* infection, the *Aspergillus*-treated plants behaving exactly as the non-vaccinated controls. It would hence appear that such vaccination affords a partial protection against subsequent infection, and the specificity of such protection from the scanty data available certainly suggests an analogy with animal vaccination.

In his monograph of 1928 (136) Nobécourt reports experiments of a similar type but using *Botrytis cinerea* and *Bacillus carotovorus*, both parasites of the bean. Sterile bean seedlings were watered with gradually increasing concentrations of extract of *Botrytis* cultures, a procedure which did not produce symptoms of suffering in the hosts. The infection experiments with *Botrytis* following this procedure demonstrated that the vaccinated plants showed complete immunity after 20 days, while the non-vaccinated controls were

severely decayed by the mold. Similar treatment of broad beans but using the filtrate of *Bacillus carotovorus* cultures produced an immunity of such a nature that the subsequently infected plants showed only mild symptoms followed by recovery when inoculated with the bacillus, while the controls showed rapid disintegration.

This type of experiment has been criticized by Hauptmann (49), who felt that plants so vaccinated were refractory to infection not because of acquired tissue immunity but because the tissues of the vaccinated plants were impregnated with the liquid of bacterial or fungus cultures and that increased resistance was due to the presence of this auto-toxic medium, i.e. containing staling products. Hauptmann's objection has been answered, however, by some convincing experiments of Carbone and Jarach (40, 44, 77). In this refutation *Phaseolus* and *Botrytis cinerea* were used as in Nobécourt's experiment reported just above. The bean seedlings were treated with *Botrytis* filtrate and subsequent infection showed that the vaccinated beans were more resistant to *Botrytis* infection than were the controls. Some of the resistant vaccinated plants were then killed by heating to 70°C, by ether vapor, and by cold (solid $CO_2$ at $-45$°C.). If the increased resistance of the vaccinated plants were due to the presence in the tissues of auto-toxic or staling substances, then the plants killed in this manner should be also resistant. If, on the other hand, the increased resistance were due to the vitally-associated antibody production, such killed plants should be quickly invaded and disintegrated by the fungus. The latter proved to be the case. The grey mold developed as strongly in the killed vaccinated plants as in the controls, and microtome sections showed an equal penetration of mycelium in both cases. We thus see that Hauptmann's objection is untenable, and, what is more interesting and relevant to the problem as a whole, that the acquired immunity displayed by the inoculated beans is a function of living tissues, alterable and lost at death. This affords a very strong analogy to the vital nature of animal antibodies and serves thus to strengthen significantly the case for true acquired immunity in plants.

There remains, however, one important objection to such vaccination experiments as the preceding which was offered by Leemann in 1932 (102). Leemann in studying the effect of various soil treatments upon the susceptibility of wheat seedlings to *Helminthosporium sativum* found that extracts and decoctions of various types of bacteria and certain fractions thereof in some cases increased and in other cases decreased the resistance of the wheat to the fungus. Among the treatments employed, Leemann found that a broth culture of dead and disintegrated *Helminthosporium* mycelium increased the resistance of the wheat, which fact if considered alone would serve as confirmatory of the experiments of the other workers reported above. Yet the protection against *Helminthosporium* afforded by extracts of *Bacillus fluorescens*, for example, indicates either that Leemann was dealing with a true antigen-antibody reaction which was highly non-specific, or that the problem of antibody production and demonstration in plants is complicated by the presence of other phenomena, perhaps analogous to staling. In other words, Hauptmann's objection, although not applicable to the *Phaseolus* experiments, may have an application when vaccination experiments as a whole are under consideration. An alternate but similar hypothesis is that the bacterial cultures may produce substances toxic not to themselves and thus not staling products in the customary sense but inhibitory to the development of *Hel-*

*minthosporium* much as Müller has found that certain inorganic fungicides and insecticides may be introduced into plants through the soil and play a part in the prophylaxis of plants (131). Leemann's work accordingly affords two important additions to our knowledge of vaccinotherapy, namely that in performing vaccination experiments we must take into consideration the possibility that the reactions may be due either to antigen-antibody effects or to the introduction of fungicidal or bacteriocidal substances in a simpler sense, another type of "pseudo-reaction," and second that in spite of this theoretical difficulty the practical application of vaccinotherapy is not interfered with but is perhaps aided, since the ultimate practical therapeutic value of vaccination is the same whether one or the other of these mechanisms is responsible.

Before leaving the subject of the active immunization of plants by vaccination we have yet to consider the experiments of Benigni regarding a slightly different aspect of acquired immunity. As was seen in an earlier part of this paper the peculiarities of plant disease may conceivably lead to different manifestations of immunity than are seen in animal immunology. As a representative difference between the two types of disease one may consider the rôle of the fungus spore in plant infections, a phenomenon having little analogy in animal immunity. Accordingly the experiments of Benigni on the effect of vaccination of *Zea mais* on the germination of *Ustilago zeae* spores are of particular interest. Miss Benigni in 1927 (12) germinated several varieties of corn seeds in water, Roulin's fluid, water and macerated smut spores, and Roulin's fluid and macerated smut spores. The seedlings in the smut extracts grew less luxuriantly than those in the pure liquids, but when later planted out these differences became less noticeable. Juices were then extracted from all four types of plant and smut spores permitted to germinate in hanging drops of such extracts. Germination was good in the plants which had absorbed only water, somewhat less in those which had absorbed Roulin's fluid, but almost *nil* in the plants which had been vaccinated. A little later, however, these differences were not apparent, and one may accordingly conclude that there is a certain grade of immunity in plants so treated expressed by an inhibition of germination of the spores of the parasite, although this immunity is partial and temporary, lasting, Miss Benigni believed, only as long as the pathological condition induced in the plant by the antigenic or toxic substances of the fungus. Leach, on the other hand, was unable to find in the extracts of rusted wheat plants substances which inhibited the germination of uredospores of *Puccinia graminis* (101).

Summarizing the results of the active immunization of plants by vaccination we may say that all of the numerous experiments thus far reported are in agreement in finding that such treatment of plants either with attenuated strains of parasite (Riker's findings excepted) or with extracts of parasite results in an increased and often very highly increased resistance of variable duration to the subsequent parasitic infection. As yet we know little about the precise nature of such prophylaxis. In some cases (Leemann) it appears to be due to a relatively simple absorption of fungicidal material. In other cases (Carbone and Jarach) it is bound to the living state of the protoplasm and is apparently of the zoöimmunitary type. The *fact* of acquired immunity by plant vaccination has been satisfactorily proved. Its *nature* awaits more critical study, but when one takes into consideration the other types of experimentation than vaccination, the

theory that acquired immunity of the zoöimmunitary type does occur extensively in the higher plants and plays an important rôle in natural prophylaxis appears to have been satisfactorily demonstrated.

Having considered the subject of the active immunization of plants by vaccination we have yet to note the very few experiments which have been performed in the *passive vaccination of plants*, i.e. in the introduction into plants not of antigenic material but of antibodies, either of plant or of animal origin. It is sometimes difficult from the nature of the experiment performed to determine whether a given experiment is illustrative of active or passive vaccination. Thus in the experiments of Sieden and Trieschmann (158), where the introduced substance was an extract of *Synchytrium* warts of potato, one is not sure whether the protection afforded was due to the antigenic stimulation of the *Synchytrium* substances (active) or to the antibodies of potato origin (passive). But there are a few cases which appear to illustrate that plants may be given successful therapeutic treatment by means of the introduction of antibodies.

Antibodies of plant origin were employed by Ray in 1901 (147), by Arnaudi in 1930 (47), and probably by Zoja in 1925 (192). The first investigator spoke of having successfully used preventive or curative antibody administrations to plants but gives us no details as to the exact nature of his experiments. Miss Zoja found that vaccination of wheat with extracts from diseased wheat plants served to inhibit later infection with *Helminthosporium*. This, however, may have been due either to an active or to a passive functioning of the vaccine as in the work of Sieden and Trieschmann. Arnaudi mentions only a single experiment of this nature in which the growth of a secondary tumor on geranium (*Bacterium tumefaciens*) was inhibited by joining to the place of the second infection a T-shaped tube of water leading to a primary gall. This evidence is accordingly very scanty and hardly serves to establish the point that antibodies of plant source may be used in the passive vaccination of plants.

In this connection should also be mentioned the experiments in which attempts have been made to vaccinate with antibodies of plant origin by means of grafting a susceptible with an immune plant. Wormald and Grubb (189) found that substances responsible for the immunity of apple to crown gall may be transmitted from immune scion to susceptible stock. In the cases of Chrysanthemum rust (Gibson, 68), the wart disease of potato (Roach, 153), and the downy mildew of potato (K. O. Müller, 130a) no such transmission of immunity-conditioning substances was observed. The experiments of Müller deserve a word of explanation because in these the basis of the work was definitely the conception of acquired antibodies in potato against *Phytophthora infestans*. Müller found that if susceptible varieties were grafted upon immune (wild) races of potatoes, and then infected, there was no alteration in the behavior of either susceptible scion or resistant stock, in the case where stock and scion were both infected more than three weeks after grafting (evidence against the circulation of normal antibodies), as well as in the case where resistant understock was heavily dosed with parasite inoculum after grafting but previous to the inoculation of the susceptible scion (evidence against the circulation of acquired antibodies). These negative results do not exclude the possibility of the activity of acquired antibodies in the cases mentioned, but resolve the question into three alternatives: (a) no antibodies are involved in the protective mechanisms under consideration; (b) antibodies are formed and passed from immune to susceptible graft-

partner but are chemically so changed in transit as to be rendered ineffective in protection; and (c) antibodies are elaborated but are not freely circulated through the plant. As will be seen subsequently, there is some evidence favoring the last of these three alternatives, but this question is assuredly worthy of more thorough investigation.

The only experiments involving the use of animal antibodies in plant therapy are those of Arnaudi (4, 6, 47) with crown gall of geranium. Arnaudi obtained a rabbit serum highly agglutinative to *Bacterium tumefaciens* and placed cut branches of geranium bearing galls in vessels containing weak solutions of such serum. After 10 days the galls in the anti-*tumefaciens* serum had withered up and shrunken while the control galls in water remained large and green. Moreover treatment with such serum followed by inoculation of fresh stems with *Bacterium tumefaciens* demonstrated a protective action of the serum against the formation of new tumors. Arnaudi's experiments involved very few plants, so few that one is not justified in drawing general conclusions from them. However, it is interesting to find that the few experiments which have been performed in the protection or cure of plants by means of the passive introduction of antibodies of either plant or animal source are all mutually confirmatory and we are thus oriented in and stimulated to investigate farther a field which from the practical standpoint of phytotherapy is no less significant than that of the active immunization of plants.

### C. *The evidence from morphology and specialization in parasitism with particular reference to the cereal rusts*

The establishment of a parasitic relationship in plants is attended by a number of visible reactions on the part of both parasite and host. Such reactions, as for example the formation and appearance of haustoria, have long been described in plant disease but only recently have they been subjected to analysis in the light of their possible immunological significance. In animal disease a few such morphological reactions of immunological nature have been observed *in vivo*, e.g., the agglutination of bacteria, but the fact that in plant disease the fungus mycelium plays a predominant rôle leads one to wish to study plant disease in this connection in order to obtain evidence shedding light upon the other features of acquired plant immunity. Observations on the morphological reactivity of host and parasite in plant disease of probable immunological significance have now been reported by numerous investigators, and have been concerned both with diseases caused by fungi and diseases or infestations due to animal parasites, and accordingly these two phases will be successively considered.

As regards morphological disturbances of plant hosts and fungus parasites the first recognition of their immunological nature was due to Bernard in 1909 (17). In certain benign and localized plant maladies Bernard remarked that one often observes hypertrophy of cells with nuclear deformity, the nuclei in some cases even becoming amoeboid. At the same time the mycelium of the parasite is variously modified in the form of suckers or haustoria which may be globular, lobulate, or even ramified or arbuscular. According to Bernard these modifications are of immunological significance: the nuclei respond to fungus stimulation by becoming enlarged, amoeboid, and taking on a function similar to phagocytes in animal immunity. The gnarled or lobulate or otherwise deformed haustoria represent reactive forms of mycelium perhaps analogous to the agglutinated masses of bacteria *in vitro* in serology. Because of the necessarily artificial divisions of the present

paper it is necessary to anticipate slightly the next subject for discussion, immunity in symbiosis, to remark that Bernard's observations on parasitism were not the product solely of his work in this field but came more or less as a corollary to his more thorough work on symbiosis, which from Bernard's point of view is merely a specialized, controlled type of parasitism. His remarks on parasitic fungi apply particularly to the Uredinales and Peronosporales, in which highly specialized types of parasitism the morphological reactivity of the fungus mycelium is particularly evident.

In the invasion of cells by fungus haustoria an activity of the host is sometimes seen in the deposition on the haustoria of encrusting layers preliminary to a dissolution of the haustoria. This has been observed in the Peronosporales by Mangin (57), in *Blepharospora*, *Peronospora*, *Helminthosporium*, and *Alternaria* by Dufrenoy (57) and in *Peronospora* by Pantanelli (140). According to Pantanelli the membrane is a gluco-proteid analogous to chitin, while Dufrenoy finds it to be of cellulose.

Fischer and Gäumann (64) have pointed out the fact that in *Giberella saubinetii* infection of Gramineae the mycelium of the parasite is morphologically normal so long as it continues in the intercellular spaces of the host, but when it enters the cells the haustoria become deformed. Mueller (130) has also observed in *Hypochnus solani* a similar deformation, corrosion, and gradual dissolution of the older haustoria. These few examples will illustrate the type of phenomenon in which we are at present interested, although they are only representative of many other such observations.

Blackman in a general criticism of the theory of acquired physiological immunity as applied to plants (23) has recognized the reaction in infected plant cells, particularly in Erysiphaceae and Uredineae, which leads in some cases to the death and even to the digestion of the invading hyphae. This death and digestion of the haustoria, however, he interprets as being due merely to the lethal and autolytic processes attending the death of the parasitized cell, since "when susceptible forms are attacked we find no spontaneous cure, no recovery of the attacked cells."

Passing from the fungus diseases of plants to infestations by animal parasites we find additional evidence regarding the acquired morphological reactivity due to the stimulus of the parasitic relation. Bernard (17) has observed in melons invaded by the eelworm *Heterodera radicicola* the formation of giant cells with fragmented nuclei resembling phagocytes. Kostoff and Kendall (94) have also seen reactions in plants infested with nematodes, particularly displayed in the form of abnormal vacuolations, which they suppose may be regulated by the continuous immunological processes occurring in the affected parts which are stimulated by the toxins of the invader. This hypothesis is also employed by the same investigators to account for the structure and development of certain cynipid galls (93), where the galls are assumed to be formed in immunological reactivity to the antigenic stimulation of larval secretions, debris of injured cells, etc., to increase in size during the period of increasing but insufficient antibody production, and to cease development when the production of antibodies is sufficient for a neutralization of the antigenic material.

There thus appears to be no doubt that in many fungus infections and even in infestations of animal parasites the parasite and host cells exhibit profound morphological reactions to the parasitism.

How may these facts best be interpreted? It will be somewhat easier to answer this very difficult question a little later when we have considered the subjects of the restriction of the parasite in the host tissue and of symbiosis. At the present time, though, we find that the evidence offers some light upon the problem at hand. Let us consider the normal course of events in an infection such as that of the stem rust of wheat, *Puccinia graminis tritici*, as described by Miss Allen (1, 2) in infection experiments on several varieties of host.

The germination of the spores and penetration of the superficial layers of the host takes place in the same manner in both susceptible and immune suscepts. Once the fungus has penetrated, however, the resulting behavior depends upon the degree of susceptibility of the host. In a congenial host numerous haustoria are formed by the solution of a fine pore in the host cell wall through which the contents of the hyphal branch flow into the host cell. At this point the plasmatic membrane of the host cell is merely invaginated, not broken. The host cells are stimulated to increased metabolic activity and the nuclei increase in size several fold, but later they collapse. In a resistant host, on the contrary, a similar process ensues until a small haustorium is formed, at which time the host protoplasm, doubtless as a result of the toxic secretions of the haustorium, collapses and dies and becomes cut off from surrounding cells by thickened contact walls. Meanwhile substances formed in the host cell diffuse into the haustorium causing its collapse and death and even that of the hypha back of it for some distance. The relative speed of development of the haustorium and of death of the host protoplast vary considerably so that in some cases the host cell may be severely damaged at the time when the fungus is not visibly harmed, and in other cases the host cell may be still normal in appearance while the fungus has succumbed. Miss Marryat (120) has observed the same two types of reaction in resistant hosts. Miss Gibson (68) in an extensive series of rust inoculation experiments found that many rusts may penetrate wholly unnatural hosts, but that after penetration the fungi are killed, the conditions "seeming to suggest that the death of the entering hyphae is not due so much to starvation as to some poisonous substance emitted by the cells."

There are several possible hypotheses to account for the immunity and susceptibility of wheat to the stem rust. The hypothesis that the spores of the rust are not able to germinate or to penetrate the uncongenial host is invalid. Many investigators have observed equal or comparable penetration of the rust mycelium into susceptible and resistant hosts. The theory that the rust dies in a resistant host because of a lack of suitable nutriment is not supported by the behavior of the mycelium which unless it is actively killed back at cell after cell in its progress is able to derive some satisfactory nutriment from the host cells. The hypothesis that resistance is due to a pre-existent toxin or unfavorable environmental condition (e.g. osmotic relationships) present in the host cell also fails to conform to the experimental findings, for in many cases the fungus can develop rather extensively before it is ultimately killed, and moreover the very complex specificity of the cereal rust resistance, as will be treated of below, renders it inconceivable that a pre-existent and therefore presumably non-specific substance or condition could be responsible for the display of immunity. We are thus forced to consider a fourth hypothesis, namely that the immunity of the cereals to rust depends upon an active production of specific prophylactic substances within

the affected cells as a result of the specific stimulation of the fungus products. Such an hypothesis has much in its favor apart from the negative findings in the other possible interpretations.

In the first place the morphological behavior as recounted above leads one strongly to the impression that immunity here is dynamic and not static, active and not passive, chemical and not mechanical. The fungus penetrates the immune host. It establishes a small mycelium. Attempts at haustorium formation, however, are eventually thwarted and the fungus dies, but not in many cases until it has formed some functional haustoria. The injury of the host protoplast is assuredly due to the production of toxins by the parasite. Were such injury merely due to the progressive withdrawal of food from the attacked cells then there would be no subsequent death of the whole mycelium, although individual haustoria might die, but the mycelium as a whole would be expected to progress and gradually involve greater and greater areas of the affected leaf. Furthermore cells at some distance from the mycelium may be variously affected; stimulated to greater metabolic activity or even plasmolyzed. Moreover, where some germ tubes lie outside the host and others inside, the latter are killed much before the former, a condition not compatible with a starvation theory (68). Thus we are safe in concluding that the primary effect of the fungus upon the host cell is a toxic effect. What is the host's response to this? There are three possibilities. The host cell could passively succumb, it could neutralize such toxins by pre-existent prophylactic substances, or it could neutralize them by specific antibodies elicited at the time by the stimulation of the toxins. The first of these possibilities is rendered very doubtful by the fact that the haustoria are not only killed but that the adjacent hyphal stretches may be killed back for considerable distances and that all of this may happen before the host cells show any symptoms of distress. The second possibility, that of pre-existent toxins, has already been refuted above, its nonspecificity and necessarily early action being inconsistent with the facts. The third possibility, that the mycelium is actively destroyed by new-formed antibodies is, on the other hand, perfectly consistent with the morphological behavior of host cells and haustoria. The haustoria may penetrate, may derive some sustenance from the host cells and not until time has elapsed for the host cells to react by immunological mechanisms do death and disintegration of the haustoria ensue. This time may be of varying duration. In some cases it is very brief and the haustorium is killed before it is very well developed, in other cases it is fully expanded before the acquired lethal effects of the host cell become evident (the mycelium in such cases occupying broad expanses of the leaf and perhaps attaining to one stage of fructification before it is ultimately killed), and in still other cases the host cell is so tardy in its production of antibodies that the protoplast dies from the toxic effects of the fungus without succeeding in exerting its direct antibody effect upon the latter, which is, however, killed indirectly due to the loss of food in the dying cell or to the autotoxic effects of the dying protoplast, or to both. In all cases, however, the haustorium dies, and the reaction is sometimes sufficiently great to be communicated back along the mycelium, resulting in death of the adjacent hyphae. Such a sequence of events is in full accord with the morphological picture in rust parasitism and serves to explain the facts as no other assumption does. But there is further evidence for

this immunological interpretation of the cereal rust resistance.

The second line of argument for the hypothesis of acquired immunity in the cereal rusts is that of host specialization, a subject which has been well treated by Fischer and Gäumann (64). The complexity of specificity in the rust diseases of plants as well as in the diseases caused by other types of parasites has long baffled students of plant immunity. There are approximately fifty biological races of *Puccinia graminis tritici* distinguished by their differential aggressiveness toward a dozen or more races of *Triticum*. There is no correlation between immunity in one host-rust combination and immunity in other such combinations in the case of wheat stem rust. A given race of the rust may attack all varieties but one or two of the wheat, or it may attack relatively few, and the variety of wheat which is immune to any given strain of the rust may be susceptible to any other given rust race. The situation is so complicated that it is wholly impossible to explain it on the basis of the assumption of simple preexistent toxins or unfavorable environmental conditions without postulating an enormous number of such substances and an equally large number of variable physiological properties in the fungi for combatting them. It is equally impossible to explain the complexity of wheat stem rust on the basis of mechanical features. But when we analyze the problem in the light of acquired immunity in combination, perhaps, with other simpler immunological mechanisms, the problem becomes somewhat clearer. Acquired immunity in animals is characterized by its complex specificity, a specificity so complex in fact that immunological reactions afford the only precise general technique for distinguishing individual proteins from one another, and the assumption of such reactions underlying the immunity display in the cereal rusts would afford an explanation and indeed the only conceivable explanation of its complexity.

Thus far we have considered the complexity of the specialization of varieties of a given host species toward strains of a given species of parasite. But the problem of host-parasite specialization is still more involved when one considers that thousands of species of parasites are restricted to a given living host species or variety, although they may be artificially cultivated upon the most diverse dead media. There is plainly some function of the living protoplasm of resistant species which prevents the development of the parasite and which is lost at death. This is further demonstrated by the facts that narcotized plants are often more susceptible to infection than before narcosis, as has been shown by Reed and Cooley with the *Heterosporium* disease of spinach (149), by Stakman with wheat rust (166), and by Bolle with the *Alternaria* disease of *Brassica* (24), and that if immune hosts be killed in such a way as to alter least their chemical properties (e.g. by formaldehyde vapor) they may be rendered favorable substrata for growth of their parasites as has been observed in lupin by Fischer and Gäumann (64), and that in such diseases as the downy mildew of potato there is a distinct correlation between metabolic activity of the host and its degree of immunity, although in the meristematic tissues the mechanical defences are weakest (K. O. Müller, 130a).

Returning now to the specific case before us, that of the wheat stem rust, we are in a position to formulate a working hypothesis as to the exact processes involved under the various conditions of immunity. The young mycelium penetrates the cells as haustorial primordia. These secrete specific antigenic substances,

toxins or proteins, which simultaneously injure the host protoplast and stimulate that protoplast to the formation of specific antibodies directed toward the fungus products in question. Such antibodies are in the form of antitoxins, neutralizing the toxins secreted by the fungus, in the form of "agglutinins" which cause a deformity of the haustoria, or in the form of lysins, disintegrating the haustoria. Any combination of these may be present. The length of time necessary for the active functioning of the host antibodies conditions the extent of parasitic development before the death of the mycelium. Meanwhile the fungus itself may be active in counter-prophylaxis, fighting the effects of the destructive host secretions by its own elaborated system of protective bodies. Here as in animals there would be a resultant of these two counter processes which would determine the success or failure of parasitism. The production of specific neutralizing substances depends upon inherited potentialities in this direction which would condition the occurrence and efficacy of antibody production. Successful parasitism would then depend upon many steps and would be the resultant of numerous conflicting forces within the plant. It would require successful penetration of the mechanical barriers of the host, ability to live in the inner environment of the host by tolerance of its normal internal conditions of acidity, osmotic pressure, etc., ability to counteract inhibitory substances either normally present or produced as a result of parasitic stimulation, and capability of utilizing the food supplies available in the host. The hosts may vary in all these characters just as do the parasites in their ability to defeat the various host defenses. Any or all of these defenses and reactions to defense may condition a given case of parasitism, and successful parasitism must be understood as a final product of many such interacting factors. In the case of rust resistance before us the evidence points to the fact that the greatest stress in the host's defense must be laid on the acquired ability to counteract the fungus secretions and at the same time to injure the invader by toxic or lethal host secretions, but one must still bear in mind that the complex situation of the wheat rust specialization is doubtless the product of other defenses operating in a supplementary way in conjunction with the acquired prophylaxis.

These findings in the example chosen of wheat rust doubtless apply broadly throughout the field of phytopathology. Similar morphological reactions and similar, if not as well understood, host-parasite specializations occur in many other types of disease, and although it is not possible at the present time to extend this analysis to other cases of parasitism it is to be hoped that the results of the analysis of this case may find a broader application in the understanding of the nature and rôle of immunity in plant disease in general.

One point which it would be well to consider in this connection, however, is the fundamental reason for the narrow host restriction of many parasitic species. Fungi vary enormously in their host ranges from such types as ***Phytophthora cactorum*** which can parasitize plants in many widely diverse families to such as the rusts which are often restricted to a single species or variety of host. Yet in the rusts many may parasitize only two species of host but these two species may be widely removed systematically from each other, as for example conifers and ferns (*Milesia*) or conifers and composites (*Coleosporium*). Surely this type of specialization cannot depend upon a condition such that only one type of suitable nutri-

tion exists for the parasite in question and that that is found only in two such widely diverse hosts. Or can the fact that such a narrowly restricted parasite as *Phytophthora syringae* (parasitizing only lilac) grows on a very wide variety of dead substrata be reconciled with the belief that only in lilac is there to be found suitable food? And that immune plants killed by formalin vapor or anaesthetized with ether or chloroform become susceptible, does this indicate a lack of suitable nutrient in the active host? On the contrary these observations all lead one to the conclusion that immunity on the whole is not due to the lack in immune species of products which can be employed by the parasite as food but to the almost universal presence in plants of substances or conditions inimical to parasitism by all but a very few species of parasites. Such defenses may at times be mechanical, they may be chemical and congenital, but when one considers the many instances on record in which reduction of the vitality of the host makes possible a parasitism we are very strongly led to the conviction that a major rôle in plant defense is played by mechanisms which are directly dependent upon the vitality of the host, the mechanisms which we are here considering under the term of acquired physiological immunity.

We may summarize the findings of the present section before proceeding to the next subject for discussion. It has been shown that in many cases of parasitism both the fungus and the host react by pronounced morphological or cytological changes. On the part of the host such changes take the form of modification of the nuclei and plastids, vacuolation, hypertrophy of the cell, stimulation to increased metabolic activity, gall formation, etc. On the part of the fungus parasite such changes are chiefly manifested by the formation of modified branches or haustoria within the infected cells. The changes in the host may be chiefly non-immunological, due to its altered metabolism. The changes in the parasite, on the other hand, are probably often of immunological significance. The attack of the host cell upon the invading mycelium may be due to substances or capabilities congenitally present in the host, such as the ability to attack the mycelium by means of phagocytosis, to encrust the haustoria, etc., or to substances elicited by the stimulation of the parasite, i.e. antibodies. One must be very cautious in drawing the line between these two types of host reaction, since it is not unlikely that certain of the host reactions described as due to an acquired immunity of the host are in reality congenital.

In the case of the stem rust of wheat which is treated in detail it seems very plain that immunity here depends in large part upon defensive reactions of the host acquired as a result of stimulation by the parasite. Moreover, extension of the analysis of wheat stem rust to a broader consideration of parasitism of plants in general leads one to the conviction derived from several types of investigation that acquired physiological immunity plays a major rôle in the phenomena of plant disease immunity and host-specialization.

### *D. Limited growth or death of the parasite within the host tissues*

It frequently happens in pathology that a satisfactory state of parasitism may be attained but that sooner or later the development and activity of the parasite are checked so that infection spreads no farther and the plant may recover. We find this particularly true, for example, in many of the leaf spot diseases of plants,

where a given fungus causes spots of an approximately characteristic size but appears to be checked in attempts to continue mycelial development beyond certain limits. In some cases such phenomena may be due to normal causes such as the restriction attending maturity of a tissue which is susceptible only when tender and succulent. In many cases, however, such restriction appears to partake of the nature of an acquired immunity, and it will accordingly be the purpose of the present section to discuss such cases.

In bacterial infections of plants Schiff-Giorgini (155) and Carbone (36) have both observed restriction or death of the parasite after a satisfactory commencement to infection. The former investigator found that in the bacterial tubercle disease of the olive the parasitized plant develops a thermolabile lethal substance as the result of stimulation by the bacteria, which, in connection with other means of defense, serves to localize the infection. Carbone also found a similar condition in potatoes infected with bacteria, where the bacterial infections were sharply delimited so that further development did not occur.

In fungus diseases of plants we have the experiences of several workers to support the same thesis. Tisdale in 1917 (172) found that both susceptible and resistant flax plants may harbor the wilt fungus, *Fusarium lini*, but while in the susceptible plants the fungus freely develops, in resistant plants it seems to be less vigorous and abnormal in its staining reactions. There are formed in resistant hosts modified corky walls which serve as mechanical barriers to the further progress of the parasite, but it is the opinion of Tisdale that these would fail to afford protection were it not that the fungus is weakened by some toxic or other chemical substance produced by the host.

Kunkel in 1918 (99) brought out a most interesting condition in cabbage club-root caused by *Plasmodiophora brassicae*. He found that with striking consistency approximately 28 unit volumes of the "clubs" are occupied by the parasite in every 100 unit volumes of tissue.

> Each club is as thoroughly diseased as is possible. The non-infected cells are apparently free of infection not because they have accidentally escaped but because of some influence which the host exercises over the parasite. There is a limit beyond which the parasite cannot go in its growth in the cabbage tissues. How this limit is maintained is a problem that remains to be solved. It may be that the spread and growth of the parasite is held in check through the development of some protective substance in the host cells. The infected cells seem to have some means of controlling the growth of the plasmodia which they contain. If we assume that this control is exercised through the production of a protective substance or antitoxin, then it is easy to suppose that this substance might diffuse out into the surrounding cells and thus render them immune to attack. Before the parasite would be able to establish itself again in a cell it would be necessary for it to pass beyond the region of immune cells (l. c. pp. 566, 567).

Moreover in rare instances Kunkel found that almost all of the cells of a club may show infection, but in such cases the plasmodia remain very small and fail to stimulate the host cells to abnormal growth. This affords a very clear indication of the active rôle played by the host in restricting the advance of the parasite as a result of stimulation by the parasite.

Dufrenoy in his studies of diseases caused by a number of types of fungi (*Chrysomyxa*, *Blepharospora*, *Colletotrichum*, *Phytophthora*) (57, 58) found that resistance to these is a phenomenon manifested by a restriction of the mycelium after it has established a nutritional relationship with the host. This restriction results in the production of small, localized lesions, and Dufrenoy finds that the restriction is accompanied by the rapid formation of

phenolic compounds at the margins of the lesions. In the downy mildew of the vine Pantanelli (140) reports that the mycelium is restricted to the intercellular spaces, and where in exceptional cases branches are sent into the cells, these are rendered non-functional by an active defense reaction of the host.

The male gametophytes and embryos of flowering plants are parasitic. The growth of the pollen tube through the stylar tissue, for example, is closely comparable with the growth of fungus mycelium in plants. It is not surprising, therefore, that observations made on pollen-tube growth and embryo development should have led certain workers to immunological conclusions with regard to this process. Ward in 1902 (183) was the first to call attention to the possible immunological behavior of maternal tissue toward the male gametophyte when he suggested that immunity of *Bromus* to *Puccinia dispersa*, a phenomenon which he believed due to specific antitoxins (184), was resembled by the factors governing fertility and sterility of the stigma to pollen. East (59, 60) and Kostoff and Kendall (88, 93) in 1929–1931 have further developed this same concept. East suggested that the secretions of a growing pollen tube may act as antigens toward the stylar tissue, which latter in developing antibodies toward the pollen tube would inhibit the development of the tube. This process would aid in explaining the sterility of stigmas to distantly related pollen (which would be more diverse antigenically than more closely related pollen) and although speculative this serves as a possible explanation of the behavior of the pollen tube within the maternal tissues. Kostoff and Kendall observed that the crossing of very distantly related species and very closely related genera often produces hybrid embryos, but these embryos die very early in development, which according to Kostoff's unpublished investigations was attributed to immunological causes.

We thus have a fairly extensive body of data dealing with the activity of plant hosts in restricting or killing their invading parasites. As yet all of the evidence available is based upon observations, no one case having been subjected to critical experimentation. We must accordingly be cautious in evaluating these data. The numerous completely confirmatory observations and the complete lack of contradictory data, however, strike one as being very suggestive, and *a priori* reasoning certainly renders it very doubtful whether any other hypothesis than an immunological one could explain the facts. We know, for example, that in all these cases the parasite first establishes itself and for a period of varying duration is successful in its parasitism. We know that ultimately this state is interrupted. The parasite begins to show symptoms of injury; it grows no farther or even dies. It is obvious that this is due to some property now present in the host but absent previously: it has been acquired. We also know that there is no evidence that the acquired capacity for restriction of the parasite is morphologically or cytologically demonstrable. We therefore assume that it is chemical. Being chemical it may or may not be of the antibody type, but it is rather difficult from our knowledge of immunity to imagine it otherwise than immunological. Careful and ingenious experiments are necessary to prove its nature. These may be impossible at the present time because of the inherently vital nature of the processes under consideration. All we can say is that the acquired immunological interpretation of these phenomena does serve to afford a satisfactory explanation of

them. No other hypothesis yet advanced serves to explain them so satisfactorily.

Before passing on to the study of the serological experiments in parasitism it is well for the sake of completeness and to avoid confusion to mention briefly another very interesting aspect of acquired immunity in parasitism which results when one considers the behavior of the rust fungi toward highly immune hosts. We find (Stakman, 166; Zimmerman, 190; and others) that there are three distinct categories of behavior of suscepts toward rusts. The rust may penetrate and successfully parasitize, a condition of susceptibility; the rust may penetrate but because of its inability to withstand the defensive reactions of the host it may perish, a condition designated as immunity; and finally the rust may establish a highly successful parasitism on a small scale toward which the host is unable to react in a defensive fashion, resulting in the rapid killing of the first cells invaded. But in this last case the death of the infected cells before the mycelium has been able to spread widely through the tissues means the sudden withdrawal of a source of nutriment to the fungus since only living protoplasm may serve in this capacity. The fungus accordingly dies, and the infection is recognizable in the leaf only as a small dead area which spreads no farther. This last type of reaction has been designated by Stakman as "hypersensitiveness." Practically its result is an immunity of the plant. Actually this "immunity" is the result of a *susceptibility* too great to make possible the parasitic relationship. As in the case of the symbioses to be considered later, a successful continuation of the relationship between vascular plant and fungus is possible only when the host is sufficiently susceptible to permit establishment of a nutritive mycelium and sufficiently resistant to prevent that mycelium from destroying the host. Here we have an apparent paradox. Acquired immunity in animals results in the removal from activity of injurious bodies. In plants the same is doubtless in general true, but in the case of the rust diseases (and in symbiosis, which is a controlled parasitism) a certain degree of acquired immunity is indispensible to successful parasitism.

This phenomenon was noted by Stakman and subsequent investigators of the cereal rusts. Mr. I. H. Crowell in an extended series of infection experiments with *Gymnosporangium* informs me that here too he has found that one often observes infection followed by death of the infected tissues with no subsequent spread of the fungus. I have also repeatedly observed it in aster species which exhibit a differential susceptibility toward *Puccinia asteris*. In the most resistant species which are attacked the infections of the fungus are represented by very small areas which are at first yellow but very shortly die. In less resistant species, however, the primary infection may attain a diameter of several millimeters and may even form functional aecia before dying. In the same way according to Crowell some species of *Malus* are so resistant that the infections become no larger than pinheads, others permit the spots to prosper to a stage in which they produce pycnia but then die, while in still others the infections may even produce aecia before succumbing.

Although this phenomenon of hypersensitiveness is closely simulative of the parasitic restriction described in the earlier part of this section, it must be differentiated from the latter because of its entirely different causation. The one appears to be a true case of acquired immunity, the other is quite the reverse. One very important aspect of the distinc-

tion of these two types of fungus localization is brought out in attempts to determine the susceptibility of host species to a given rust. In such attempts a clear distinction must be made between hypersensitiveness and partial or restrictive immunity, because theoretically they are diametrically opposed, and because practically attempts at breeding for resistance might not succeed if one fails to consider that a potential parent species may be immune either because of a strong defensive ability or because of such great susceptibility that the fungus cannot survive within its rapidly dying tissues.

### E. *Acquired serological reactions in parasitism*

Acquired immunity in parasitism may be displayed in a number of ways. We have already considered several of these, namely immunity to reinfection, effect of vaccination, morphological changes, and restriction or death of the parasite within the host tissues. While it has been possible from a consideration of the evidence from these sources to establish the conception of acquired immunity in parasitism with comparative certainty, the final, and from the zoöimmunitary point of view the most important type of evidence is that yielded by serological tests. A complete proof of such acquired immunity requires evidence of increased resistance *in vivo* which is proven by serological tests to be due to antibodies. In animals this dual proof is a comparatively easy matter, but the reverse is true of plants. We have already seen the great difficulties besetting a serological study of plant materials. Nevertheless a number of experiments in this field have been performed and their results are of interest in concluding our discussion of acquired immunity in parasitism.

The first tests of this nature were performed by Schiff-Giorgini in 1905 (155) working with the olive tubercle disease. Pieces of wood from points near an infection were dropped into bouillon cultures of *Bacillus oleae* and led to agglutination and lysis of the bacteria, while control tubes remained unchanged. This production of protective substances was apparently localized about the lesions and did not confer an immunity on the plant as a whole, since each year the number of tubercles increased.

Mention has been made of Leach's experiments in 1919 (101) in which he was unable to demonstrate in juices of rusted wheat plants substances inimical to the germination of rust spores. This is not a situation strictly comparable to animal serological reactions, however. Such a type of reaction might be immunological in plants but the absence of inhibition of spore germination does not imply absence of any immunological control in such cases. Indeed we have already seen that in the cereal rusts acquired immunity is not expressed until long after the spores have germinated and then only within the living cell. Leach's results are accordingly of interest but they do not seriously handicap the acquired immunological interpretation of wheat rust resistance.

In 1923 Carbone performed some experiments in the demonstration of agglutinins and lysins in potato decays caused by several species of bacteria (36). After having introduced the bacteria into potato cavities for several days he expressed the sap from portions of the tubers and tested this sap against suspensions of the bacteria but with wholly negative results in every case. Carbone's experiments were carefully controlled and his tests were performed with strict adherence to the approved methods of animal serology. The argument that potato tubers are quiescent organs and lack the activity of green

tissues was refuted by Carbone's observation of the rapid and active cicatrization of the potato wounds, but he felt that possible failure in absorption of antigenic material might have accounted for his negative findings.

The following year Kořínek (81) continued this type of investigation using as experimental material crown gall of *Beta* (caused by *Bacterium tumefaciens*) and bacterial infections of *Euphorbia* and of Leguminosae. With the legumes and beets a technique similar to that of Carbone's was used, but with *Euphorbia* the latex was tested for its antibody content. The inoculated beet sap strongly agglutinated *Bacterium tumefaciens* but equally agglutinated *B. prodigiosus*, and the non-inoculated controls also showed strong normal agglutination. Accordingly his results with beets are not to be considered of immunological significance because of the error due to the strong normal agglutinins. The agglutination observed was therefore termed a "pseudo-agglutination" both because of its non-specificity and because microscopic sections showed it to be doubtless due to a chemotactic action of crystals present in the root sap. The *Euphorbia* latex, on the other hand, was found to contain neither normal nor acquired agglutinins toward inoculated bacteria of several species, and the same was likewise true of various inoculated species of legumes. Kořínek's evidence is accordingly wholly negative. What "antibodies" were found proved to be non-immunological in their causation and behavior.

Sardiña in 1926 (154) performed some carefully controlled and executed experiments for the demonstration of antibodies in a number of types of plant infections. In the case of *Opuntia* infected with the potato blackleg bacterium the inoculated plants showed acquired precipitins while the non-inoculated controls were negative, but in all other cases (*Opuntia*, *Lycopersicum*, *Cucurbita*, *Solanum*, with several species of bacteria) acquired precipitins and lysins were not found. Sardiña was accordingly led by his negative results to the conclusion that species-specific agglutinins and precipitins are not formed in these species and that in this respect plants differ from animals and man. Technically Sardiña's work gives every evidence of care and thoroughness and may be regarded as a satisfactory demonstration of the results obtainable by the expressed-sap method.

Negative results were also obtained by Riker in 1926 (152) using the same technique. The expressed sap of plants bearing crown galls was found to exert no agglutinating, precipitating, or lytic action upon suspensions of *Bacterium tumefaciens*. That antibodies might have been produced in the galls but not circulated was apparently argued against by the fact that the bacteria grew as well in the expressed sap from the galls as in the expressed sap of non-infected controls. Riker accordingly came to the conclusion that either antibodies are not produced in crown-gall or else they are in insufficient quantities to be measured by ordinary methods.

To the experiments reported above should be added mention of East's experiments with sugar cane mosaic in 1931 (61). East found in a series of "precipitin tests" of expressed sap of various types of sugar cane that there is some indication of a consistent reaction difference between cane which has never had the disease and cane which has or may have had the disease. This evidence, though acknowledged by East to be very slight, appeared at the time to be serological evidence supporting the thesis that sugar cane recovers from mosaic by an immunological process.

Beside these serological tests of diseased

plants one must also make mention of the serological interpretations advanced by certain authors to explain the reactions of plants to infection. Thus, for example, Heinricher felt that the acquired resistance of pear to mistletoe could only be explained on the assumption of a toxin-antitoxin reaction (71, 72), Steiner considered that the specialization of the nematode diseases of plants is explainable by the same hypothesis (167), and Kostoff and Kendall also advance a serological theory to account for the reactions of Solanaceae to nematode infestations (94).

We thus see that the problem of the demonstration of acquired immunity in plants by serological techniques has been attacked by numerous workers. Their experimental results may be grouped into three categories: (a) demonstration of non-immunological pseudo-antibodies (East, Kořínek); (b) demonstration of no acquired serological reactions (Leach, Carbone, Kořínek, Sardiña, Riker); and (c) apparent demonstration of the acquirement of antibodies (Schiff-Giorgini).

Regarding the first class of reactions Kořínek has shown that his positive results are not only non-specific but that they are due to a simple physico-chemical mechanism, chemotaxis. East's technique has since been studied by Chester and Whitaker (52, 53) and although the results at first are strongly suggestive of an immunological character, the analysis of these latter investigators has shown that the "precipitin reaction" technique of Kostoff, East, and others is in no case observed immunological, but that the reactions observed are interpretable in the terms of simpler non-immunological chemistry.

As concerns the second group of experiments, it would seem that the technique of testing of the expressed sap against pathogenic bacteria has been satisfactorily demonstrated to reveal no antibodies. We have the evidence of five careful experimenters all mutually confirmatory in finding that the expressed sap of the infected tissue fails to exert immunological action upon the parasite involved. Against the results of these investigators those of Schiff-Giorgini stand in isolated opposition, alone and unconfirmed. The evidence of Schiff-Giorgini can accordingly be given little weight in comparison with that of the other investigators. Either his technique or material were more favorable in some unrecognized manner, perhaps due to the fact that he used pieces of infected tissue and not expressed sap for the source of antibodies, or else he was dealing with reactions of a "pseudo" or normal type, and it seems likely from the thoroughness of the subsequent experiments that the latter of these suppositions is the correct one.

If then we are to conclude that all of the serological tests in parasitism are either non-immunological or negative we are confronted by a paradox demanding explanation, for certainly the numerous observations *in vivo* point definitely to an acquired immunity in parasitism. How may this apparent paradox be explained?

There are certain marked differences between the techniques of animal serology and those which have thus far been employed in plant work. Chief among these is the very considerable change which must occur in the expression of sap from living cells. It has already been shown in several instances that acquired immunity in plant parasitism is a function of the *living cell*. Anaesthesia or death by the most careful processes may render immune plants susceptible, and if such immunity is due to the action of vital antibodies the technique of tissue maceration, the death of the cells, the mixture of many substances which in nature are separate, and the irreversible modification of many others must necessarily lead to the

final obtaining of a sap or extract which differs very fundamentally from the fluids of the living cell. This fact has been recognized by the various investigators who have attacked this problem. Carbone has attempted, but unsuccessfully, to avoid it by employing for testing the circulating fluids of the plant (xylem and phloem currents). But thus far no one has been able to use *in vitro* a fluid from plants comparable for serological purposes with the animal blood. We are at an *impasse* in this direction. It may be that it will not be possible by zoöimmunitary techniques to add to our analysis the final serological proof. It may be, on the other hand, that a satisfactory technique can be devised. Perhaps methods of micro-manipulation or the use of large unicellular organisms such as *Valonia*, *Halocystis*, or *Caulerpa* may afford the answer. Meanwhile, however, it seems that, in view of the very strong evidence for acquired immunity in parasitism which has been derived from several sources, and in view of the profoundly atypical conditions surrounding the negative serological tests, the latter do not present an insurmountable barrier to the thesis under discussion. It would be highly enlightening and satisfying if the serological tests did conform to the experiments with living plants, but that they do not does not offer a significant refutation to the extensive evidence secured from studies in reinfection, morphology, host-specificity, and parasite localization as discussed in the earlier parts of this critique of acquired immunity in parasitism.

## VIII. ACQUIRED IMMUNITY IN SYMBIOSIS

We have seen from the discussions of the foregoing sections that the theory of acquired immunity in plants is derived from observations and experiments in the injection of plants with foreign bodies and in the behavior of plants in parasitism. Symbiosis, which from one point of view is a controlled and non-injurious form of parasitism, affords a final source of evidence upon this theme, and indeed much of the earliest and strongest evidence has resulted from such studies as those of Bernard on the orchid symbiosis and of Cappelletti on the symbiosis in leguminose root tubercles. First, both in order of time and in immunological importance, will be discussed the results of the orchid studies, and this will be followed in turn by consideration of the symbiosis in the root tubercles of legumes, of other natural symbioses, and of the artificial symbiosis of the graft-union, all of which contribute to our knowledge of acquired immunity in plants.

### *A. Symbiosis in the orchid mycorrhiza*

For fully fifty years the peculiar behavior of the mycelium of orchid mycorrhiza in the host tissues has been described and discussed. As early as 1886 Wahrlich (181) came to the conclusion that "the yellow clumps which are found in the root parenchyma of orchids are not plasma balls and do not belong to the host tissue but to the orchid fungus, and are true haustoria, later surrounded by hyphae." The significance and fate of these balls of hyphae within the host cells were not further understood at this time, however.

Wahrlich having noted the presence and distortion of the fungus mycelium within the orchid cells, it remained for Dangeard and Armand in 1897 (55) to call attention to the fact that the host cell is not passive during its invasion but that its nucleus becomes amoeboid, being active in passing from the ball of hyphae in the center of the cell to the periphery of the cell where it may assume various forms. Our first suggestion as to the immunological proc-

esses in the orchid mycorrhiza is due to Magnus who in 1900 (108) found that there are in the orchid two reactive types of cells, "host cells" in which the fungus never degenerates but which appear to serve as sources of nutrition for the mycorrhiza, and "digestive cells" in which the fungus first enters as normal haustoria which latter eventually become gnarled, clumped, skein-like, and finally die and are dissolved or digested. Meanwhile the host protoplast is not killed although it may become highly vacuolate, and the host nucleus may become hyperchromatic and amoeboid.

Bernard's memorable series of studies commenced with an analysis of the rôle of mycorrhiza in tuberization in 1902 (13). He determined that there are three types of tuberization: precocious and permanent tuberization with mycorrhizal infection at the beginning of seed germination and extending throughout the life of the plant as in *Neottia*, precocious and periodic tuberization with infection at the time of germination followed by alternate periods of tuberization (with mycorrhizal infection) and non-tuberization (without mycorrhizal infection) as in the *Ophrydeae*, and late and periodic tuberization such as occurs in the potato (wild) with infection and the resultant tuberization occurring only at maturity. Bernard concluded from these studies that tuberization is the result of mycorrhizal infection and that in its action the fungus may act at a considerable distance through the agency of soluble products. Further early descriptions of the behavior and function of mycorrhiza apart from those of direct immunological interpretation are to be found in such works as those of Gallaud in 1905 (65) and Burgeff in 1909 (28).

In 1904 appeared the first work of Bernard dealing directly with immunity in the orchid mycorrhiza (14). He found that among orchids *Bletia hyacinthina* is anomalous in that the embryo at first shows an almost complete immunity to the mycorrhizal symbiont, while later it not only becomes capable of infection but will grow no farther unless infected. The immunity takes the form of a precocious and very active digestion of the hyphae, which are able to enter the tissues unimpeded. By 1908 (15) Bernard had succeeded in obtaining non-virulent strains of the mycorrhizal fungi, which strains if inoculated into the orchids could invade the tissues but could not cause germination.

The following year in two very important papers (16, 17) Bernard reached the culmination of his studies on acquired immunity in the orchid mycorrhiza. His findings may be briefly summarized in the following terms. Mycorrhiza in the orchids has probably evolved from an active parasitism in the past. The fungi involved belong to genera characterized in many cases by virulent parasitism (*Nectria*, *Hypomyces*, *Rhizoctonia*). On the first entry of the fungus there is a rapid and unchecked invasion of the host tissues. This is soon opposed, however, by an acquired immunity which lasts for some time and inhibits secondary infection. If the first fungus inoculated is capable of forming a permanent association with the orchid, this resistance to reinfection is permanent, but if it is not adapted to such a consortium, the period of resistance to reinfection is only temporary and a second infection may occur. The immunity displayed by the orchid in checking and controlling further spread of the fungus within its tissues is of two sorts, a normal phagocytic immunity and an acquired humoral immunity. The phagocytic immunity is displayed particularly by certain specialized cells which lie in the deeper parenchyma

and in which the lobed nuclei are able to surround and digest the progressing mycelium. The humoral immunity, which is acquired as a result of the stimulation of the advancing mycelium, results first in a clumping or gnarling of the hyphae within the cells, which hyphae subsequently become dissolved or digested. The acquired immunological nature of this humoral immunity was demonstrated by experiments *in vivo* (18). Pieces of infected orchid tuber if placed upon sterile agar secreted substances which diffusing out through the agar not only checked the advance of a pure mycelium of the mycorrhizal fungus upon the agar, but even killed it back. Older tubers showed a more abundant production of the controlling substance than younger tubers. The toxic substance was thermolabile since it lost its toxic effect on heating to 55°C for 35 minutes. A certain degree of specificity was displayed by this diffusable substance since its action was directed in the main against the specific fungus infecting the tuber.

Bernard's work was confirmed in 1923 by Nobécourt (134), who repeated Bernard's experiments on the diffusability and nature of the humoral substance secreted by infected orchid tubers. He again observed the controlling and checking action of such a diffusable substance and further tested the question of whether the toxic substance pre-existed in the tuber before placing it on the agar. This was accomplished by subjecting the tubers to cold (−15°C) and chloroform vapor. Such a process decreased the reactivity of the tuber and the effect was believed by Nobécourt to be upon the cell and not upon the antibodies, which latter he therefore assumed were not pre-existent in the cell. But Magrou in 1924 (113; see also Magrou, 112 and 114) asks the question: Why were not antibodies pre-existent in the cells since the cells were previously infected by the mycorrhiza? His answer to this question was obtained by placing a piece of tuber for two weeks on an agar slant and then removing it and planting the slant with the specific fungus, *Rhizoctonia* sp. The result was that the fungus grew very poorly and then in a direction opposite to that in which the tuber fragment had lain. Magrou therefore concluded that the anti-mycorrhizal substance first demonstrated by Bernard and later confirmed by both Nobécourt and himself was pre-existent in the infected tuber, as would necessarily be the case in a true acquired immunity in the orchid mycorrhiza.

Bernard's findings on the morphology of the orchid mycorrhiza and his conclusions as to the acquired immunological nature of the orchid immunity have also been supported by the experiences of numerous others, among whom should be mentioned Ramsbottom (144), who confirmed the effects of vaccination of orchid tubers although he failed to observe phagocytosis, and Magrou (111–114, 116, 118).

We thus see that there is a complete body of evidence, repeatedly confirmed by various investigators, regarding the rôle of acquired immunity in the orchid symbiosis. It is plain from this evidence that the orchid tissues oppose the progress and potential parasitism of the fungus symbiont by means of two mechanisms, one a normal property of the host cell (phagocytosis), the other an acquired humoral mechanism in which, due to the stimulation of the fungus secretions, noxious and inhibitive and even lethal specific substances are produced by the host. These substances possess the properties of animal immune bodies, and their presence and nature have been determined by experiments both *in vivo* and *in vitro*. We may accordingly conclude that here in the orchid mycorrhiza we have a complete and

satisfactory proof that plants may show a defense mechanism homologous with that of animal serology.

*B. Symbiosis in leguminose root tubercles*

The study of immunity in the root tubercles of Leguminosae has likewise been extremely productive of evidence supporting the thesis that plants may show acquired immunity of the zoöimmunitary type. Here, as in the preceding case, such evidence is both morphological and serological.

It has long been known that the root tubercles of legumes contain symbiotic bacteria which on first infection are normal but which as the tubercles grow larger assume various atypical forms, passing through a pre-bacteroidal stage of swelling to a bacteroid stage in which they assume X and Y and arbuscular forms and are greatly enlarged. The immunological significance of this was first pointed out by Hiltner and Störmer in 1903 (73, 74). These investigators came to the conclusion that the reactions of host and bacteria are best explained in the terms of a partial or controlled active immunity, this conclusion being based upon the facts that in the expressed sap of leguminose plants there is a substance which when added to bacterial colonies causes plasmolysis and disintegration of the bacteria, and that inoculation with a virulent strain of bacteria prevents the possibility of later infection with less virulent bacteria.

The theory of Hiltner and Störmer was confirmed in principle, although criticised in certain details of the interpretation, by Süchting the following year (169). Süchting likewise recognized the acquired immunological character of the relationship here as may be seen from his remark that "there is here in plants a production of prophylactic substances, antibodies, just as the bacteria form toxins or infectious substances." He further supported the theory of acquired immunity in this symbiosis by his observation that there is a definite limit to the number of tubercles which may be formed, regardless of the amount of inoculum introduced, this limit being conditioned by an immunological equilibrium within the plant. This is a situation strikingly reminiscent of that found by Kunkel in cabbage club-root as described above.

For the most complete and extensive work on immunity in the leguminose root tubercles we are indebted to the studies of Cappelletti in the years 1923–1928 (29–34). Cappelletti made a careful investigation of the morphology of the tubercle bacilli in comparison with the serological behavior of the host extracts. He found that in their reactions toward the bacteria the Leguminosae comprise two distinct types. In one type, typified by *Pisum*, bacteroids are formed accompanied by nuclear disturbances and the production of antibodies in the tubercles specifically directed against the bacteria. In the other type, typified by *Phaseolus*, bacteroids are not found, and neither are antibodies demonstrable in the tubercle saps. The antibodies found manifest the usual thermolability of animal antibodies, being destroyed by heating for one-half hour at 78–80°C. There is a clear correlation between morphology of the bacteria and serological reactivity of the host. Bacteroids are present in the tubercles only at maturity of the plants, from the period of inflorescence on. Agglutinins and precipitins are likewise present only at this time, being absent in the younger tubercles which contain no bacteroids. If inflorescence is prevented one observes a new development of tubercles in which agglutinins are evident although not abundant. If the plant is then left alone new flowers are formed, complete bacteriolysis occurs,

and there is a rapid and notable augmentation of the agglutinins. The antibodies formed appear to be strictly localized in the infected cells. Thus if the tubercle bacteria occur in the intercellular cavities in the tubercles they fail to assume the bacteroidal form (Dangeard, cited without reference in 32).

Cappelletti's work shows that there is a complete and harmonious correlation between the abnormal morphology of the bacteria and the presence of antibodies of the zoöimmunitary type. Here as in the case of the orchid mycorrhiza the work is relatively complete although there are still a few perplexing questions to answer. Among these may be mentioned the lack of conformity between Cappelletti's theory of localization of the antibodies and Süchting's finding regarding the inhibition of new infections by pre-existing tubercles. Moreover Kořínek (81) was unable to demonstrate agglutinins in root tubercles of Leguminosae, perhaps because he was dealing with Cappelletti's *Phaseolus* type, or because he was testing at a period before the appearance of the antibodies, i.e. before flowering. Němec, on the other hand, has observed agglutination or a phenomenon resembling it *in vivo* in leguminose root tubercles (132). Another problem is raised by the discovery of Zipfel (191), Barthel (8), and others that the bacteroidal form may be induced in artificial culture by the addition to the culture media of such substances as caffein, glucosides, certain carbohydrates, inorganic substances such as saltpeter and phosphates, and organic acids and their salts. This does not necessarily contradict Cappelletti's theory, since it is well known that the various types of serological reactions in animals may be simulated or reproduced by non-immunological causes, but it requires that one exert his utmost caution in deciding whether a given reaction is or is not immunological.

However, the burden of Cappelletti's argument appears clear and complete. His observations *in vivo* are thoroughly supported by his serological tests, and the latter give evidence that he was dealing with reactions of the zoöimmunitary type. Accordingly, we may with safety add the case of the leguminose root tubercles to that of the orchid mycorrhiza as a second sound body of evidence supporting the thesis of acquired immunity in symbiosis.

### C. *Other natural symbioses*

No other natural symbioses have been studied from the immunological viewpoint with a fraction of the thoroughness with which the orchid mycorrhiza and the Leguminosae have been. Yet numerous other types of symbiosis do occur in nature and these have in many cases been studied morphologically at least. It is accordingly instructive at this point briefly to report such findings in these other natural symbioses as relate to the investigations discussed above.

The mycorrhizae of Solanaceae have been somewhat more thoroughly investigated in this connection than symbioses in other groups and served as the material which led Bernard to his theory of the dependence of tuberization upon symbiosis (13, 19). In solanaceous tubers Magrou (109, 110, 113, 115) found present the same type of phenomena as have been found to be of immunological significance in the orchid mycorrhiza, such as the clumps of mycelium within the host cells which were later digested by the host protoplast. Phagocytosis was also observed within the cells, but this normal mechanism of immunity, according to Magrou, seemed insufficient to control satisfactorily the invader, and the localization of the mycorrhiza in the host tissues appeared to be the result of an acquired humoral immunity comparable to that in orchids. Magrou's experiments have been criticized

by Ducomet (in Magrou, 113), who claimed that Magrou had used too few plants, plants of variable stocks and unsuitable age, and incorrect interpretation of numerical data. Magrou's answer to this criticism of Ducomet, however (113), appears to justify Magrou's work.

The clumping of mycorrhiza mycelium followed by its death and digestion by the host as observed in orchids and Solanaceae has been likewise seen in a variety of other symbioses. Janse in 1897 (76) described such hyphal abnormalities in a wide variety of mycorrhizae including hepatics, Lycopodiales, Selaginellales, ferns, gymnosperms, monocotyledons, and dicotyledons. Shibata in 1902 (157) extended the same findings to include *Podocarpus*, *Psilotum*, and *Myrica*. Similarly clumping of the mycelium, phagocytosis, and digestion have been observed in hepatics by Gallaud (65) and Magrou (117), in *Arum* and *Paris* by Gallaud (65), in *Calluna* by Rayner (148), and in *Lathyrus* and *Mercurialis* by Magrou (111). Magrou's studies on *Pellia epiphylla*, an hepatic, are of particular interest, since here the observations are in considerable detail and appear to show that the localization of the mycorrhiza within the host thallus is controlled by fungicidal substances secreted by the reproductive portions of the plant. Active phagocytosis occurs but in addition to this there seems to be an active humoral immunity which checks the growth of the mycelium as it grows toward the sporogones and archegones.

Finally in lichens analogous phenomena have been recorded by Moreau (123, 124). In *Ricasolia* it frequently happens that algae (Cyanophyceae) not belonging normally to the lichen thallus come in contact with the lichen thallus, which forms about them tubercles known as cephalodia. These may be either internal or external and represent a temporary, unstable symbiosis or parasitism in contrast to the normal, permanent lichen symbiosis. Within the cephalodia the foreign Cyanophyceae are gradually killed and dissolved, a process compared by Moreau to the killing and lysis of foreign bacteria in the animal body, and attributed to a defense reaction presumably of the type in which we are here interested.

We thus find that the orchid and lichen symbioses are by no means isolated cases of acquired immunity in symbiosis. Comparable or identical phenomena occur throughout a very wide range of plants, including all of the four major plant subkingdoms. It would thus appear that acquired immunity of the type demonstrated in the higher plants is a very widespread phenomenon, an attribute of living matter at all levels of plant as of animal life. Such a conception is based to some extent upon analogy, but it would seem that the analogy is justified in view of the large number and wide range of perfectly confirmatory observations. That acquired immunity of the zoöimmunitary type plays a fundamental part in the control of the potentially parasitic symbiont is manifest in both cases which have been studied in detail, and in conjunction with all the other types of evidence preceding offers a final proof that the ability for an organism to respond to the stimulus of foreign bodies by acquired humoral reactivity is an attribute not of the higher animals alone but of life in both kingdoms.

### *D. The graft-symbiosis*

The graft union is an artificial form of symbiosis, since stock and scion are mutually interdependent. In the graft union two tissues of different origin are brought into intimate mechanical and physiological contact. It might therefore be expected that the graft union would offer an excellent opportunity for studying acquired immunity, since each graft-symbiont would be expected to sensitize the other in an

antigenic manner leading to the elicitation of antibodies in each symbiont against the other. This conception was made the basis for an extensive series of investigations by Kostoff in 1928–1931 (82, 84, 86, 87, 88, 90). This type of work has been subsequently investigated by Silberschmidt in 1931 (161) and by Whitaker and Chester in 1932 (187).

Kostoff's experiments were performed with numerous genera and species of Solanaceae, which family is well adapted to graft and cytological techniques, and they included morphological, cytological, and serological findings which appeared to be mutually confirmatory in demonstrating the presence of antibody-formation in such grafts. Kostoff's morphological evidence of acquired immunity in his grafts included his observations that repeated grafting upon the same stock is not possible since although a first scion may successfully graft upon a given stock, a second grafting of a similar scion upon the same stock may be attained with difficulty or wholly inhibited, that the grafted plants frequently showed morphological malformations such as galls, and that grafted scions often showed abnormal corollas and calices. His cytological evidence comprised the finding of irregular and abnormal meioses, the failure of starch to cross the graft union, and agglutination of the plastids of cells near the graft union (83, 84, 87). This morphological and cytological evidence was supported by two types of serological investigation, namely investigation of the mutual precipitin potency of extracts of stock and scion (as determined both by gross reading of ring tests, and by the more delicate nephelometric method of measurement) and of the mutual lytic activity of stock and scion (as determined both by gross observation of lytic rings and by the dialysis-Ninhydrin technique which is based upon a chemical, colorimetric determination of the amount of protein-cleavage-products before and after lysis). These various types of serological investigation were found by Kostoff to agree in demonstrating that in a few of his graft unions there was an increase in precipitin and lytic potency of stock toward scion and of scion toward stock after grafting, an increase which was strongest near the graft union, weaker farther from it, which increased to a maximum at about one month after grafting, and which exhibited a partial but imperfect specificity. Kostoff believed that his serological results served to explain the morphological and cytological results, and that taken together these offered a complete demonstration of acquired immunity in grafted plants.

Silberschmidt in an excellent discussion has reviewed Kostoff's work and offered numerous suggestions based upon the assumption that Kostoff's reactions were truly immunological protein reactions. Silberschmidt, however, in his few exploratory grafts failed to obtain any significant rise in precipitin potency due to grafting.

In a second contribution (161a) Silberschmidt has reported further investigations of the Kostoff reactions. Although some positive results were obtained, the "acquired precipitins" found were not specific, and because his investigations gave no foundation for the assumption of the existence of specific toxic substances in the grafted scions, Silberschmidt challenges the theory of the gradual immunization of the scion by the stock, at least for the graft combinations investigated by him.

Meanwhile Chester and Whitaker (51, 52, 53) had been engaged in a study of the nature of the reactions believed by Kostoff and Silberschmidt to be serological. Their study showed the "precipitin" reac-

tions in the Solanaceae to be non-immunological in character, due to non-protein substances normally present in the cell, while such observation as they made of the "lytic" reactions (ring tests) revealed the occurrence of such lytic rings in non-immunological extract layerings. Continuing their work these same investigators next studied the behavior, morphological and serological, of grafted Solanaceae (187) and found that in none of the cases tested was there any significant alteration of the "precipitin reaction" after grafting, nor was there observed any production of the morphological and cytological abnormalities reported by Kostoff. The work of Whitaker and Chester was more extensive than that of Kostoff as regards the number of species grafted, the number of grafts tested, and the variation of conditions attending extraction and testing.

It therefore appears that this technique of investigation which looked so promising is not adapted in its present form to the study of acquired immunity in plants. The "precipitin" tests which have been found in normal plants are non-immunological, the acquirement of true precipitins after grafting has not been demonstrated, and the gross observations of the "lytic" reactions have shown them to be either non-immunological or at least simulated by non-immunological reactions. There remain of Kostoff's serological investigations his dialysis-Ninhydrin results which have as yet not been repeated by other workers and which for this reason and in view of the "pseudo" nature of all his other reactions are to be accepted for the present only with reservation. Moreover his findings with regard to the morphology and cytology of the grafts have not been substantiated by others. The effects described by Kostoff, gall-formation, abnormal meioses, corolla and calyx deformity, etc., are highly suggestive of environmentally conditioned characters rather than specific antigen-antibody effects. The inability to regraft after a preliminary grafting may well have been due to the greater maturity of the tissues at the time of the second operation, since Whitaker and Chester report that grafting of the Solanaceae is much more easily accomplished with succulent young tissues. The agglutination of plastids *in vivo* may have been immunological; it may equally well have been due to a variety of other causes, such as cytological methods or nutritional disturbances about the graft union. In a word, there appears to be little or no sound evidence to support the contention that acquired immunity has thus far been demonstrated in grafting.

The failures in demonstrating this point, however, are not detrimental to the conception that acquired immunity does occur in plants. They are instructive in showing us the limitations of our present techniques and the dangers of jumping to hasty conclusions on the basis of imperfectly understood observations. Just as the expressed-sap method has failed to reveal antibodies in parasitism, so for the same reason does it appear to be unsuited to a study of acquired immunity in the graft union. It may be that refinements of the technique will reveal the possibility of demonstrating antibodies in grafted plants, or it may be that sensitization in such cases is far less effective than in parasitism and in other forms of symbiosis, so that there is little or no production of antibodies in such cases. This last would not seem unlikely when one considers the success of many interspecific grafts.

Closely allied to the subject of acquired immunity in grafting, although not dealing precisely with symbiosis, is the subject of hybridization. In hybridization there is the bringing together of two foreign protein systems, and it might be that

here acquired immunity could play a part in the success and subsequent behavior or failure of hybrids. This is a problem to which Kostoff again has contributed (84, 85, 89). He found that certain species hybrids were characterized by tumor formation, and that this tumor formation was correlated with a "precipitin" difference between the parents. Unfortunately, however, the results with the "precipitin" tests are subject to the same criticism as that applying to his grafting experiments, and since there thus remains no proof as to the serological difference between the parents the correlation with tumor formation is not significant. Likewise, and for the same reason, Kostoff's contention that serological differences may serve to explain the appearance of cancer in humans is as yet without basis from the standpoint of plant serology. Again we may say that this offers no proof that immunological phenomena may not play a part in the success and behavior of plant hybrids; we may merely conclude that such has not yet been demonstrated.

In concluding the present section we may briefly recapitulate the findings of our analysis of acquired immunity in symbiosis. We have seen that there is clear-cut and abundant evidence that the orchid controls the distribution and activity of its mycorrhizal symbiont by the production of antibodies of the serological type. There is likewise good evidence that the same applies to the action of Leguminosae toward their symbiotic tubercle bacilli. A survey of the occurrence and nature of symbiosis in many of the other plant groups including Thallophyta, Bryophyta, Pteridophyta, and Spermatophyta shows that although the complete proof has not been offered in other cases than those of the orchid and legume symbioses, yet in all these other types of symbiosis phenomena closely analogous to those of the orchid and legume symbioses occur, and it therefore seems probable that acquired immunity as observed to control the orchid and legume symbioses also occurs generally in symbioses throughout the plant realm. On the other hand, the investigations of acquired immunity in graft unions and hybrids of the Solanaceae have proved that these are not adapted to the demonstration of antibodies according to our present techniques, although this is by no means detrimental to the significance of the other findings in this paper regarding the occurrence of acquired immunity in plants.

## IX. THE SIGNIFICANCE OF ACQUIRED IMMUNITY IN PLANTS

Having completed an analysis of the experimental findings in the field of acquired physiological immunity in plants we are now able to turn this knowledge to account by a study of the theoretical and practical significance of these findings. The present section will therefore treat of the significance of acquired immunity in plants with emphasis in turn upon the proof of the existence of acquired immunity in plants, the nature of this immunity, its rôle in phytopathology with particular regard to the part played by acquired immunity in the evolution of parasitism, in epidemics and natural control, and in host-parasite relationships, the practical applications of acquired immunity in phytopathology, and the direction of future research in this field.

### *A. The existence and nature of acquired physiological immunity in plants*

The main question which has concerned investigators in this field has been: Does acquired immunity of the animal type occur in plants? The present analysis has yielded a definitely affirmative answer to this question. There are a host of experi-

ments and observations regarding parasitism and symbiosis which have led clearly to the conviction that plants may protect themselves from foreign invaders by the elaboration of specific substances directed against the intruder. The bases for this conception are the facts as demonstrated above that plants recovering from a first attack of a parasite show a resistance to a second infection by the same organism, that experiments in both active and passive vaccination have revealed in practically all cases the protection afforded by such treatment, that the behavior of the parasite within host tissues, the host-specialization of plant parasites, and the localization of parasites within their host are all better interpreted as antigen-antibody effects than in any other manner, and that the experiments on various types of symbiosis have proven that the types of symbiosis studied in detail are indubitably made possible by an acquired immunity of one symbiont toward the other, while the evidence regarding other symbioses in plants shows that this condition of acquired immunity in symbiosis is of very general occurrence throughout the whole of the plant realm.

With the exception of the studies on symbiosis the evidence for the acquirement of physiological immunity in plants has been almost entirely drawn from experiments and observations with living plants. Serological tests *in vitro* have with this exception yielded either negative or conflicting results. To the zoöimmunologist this might appear an insurmountable objection to our thesis. But the foregoing analysis has shown that we cannot unreservedly transfer all of the principles and techniques of animal serology to the field of plant immunity. We must devise new methods of attack and must be awake to the possibility of distinct manifestations of acquired immunity in the plant realm. It appears that there is one important barrier, not to the principle of acquired immunity in plants but to the application of animal methods to plants, namely the lack in plants of a circulating stream analogous to the blood stream. In both kingdoms the living cell is undoubtedly the reactive unit responsible for the production of antibodies. In animals in addition there is the blood stream, which serves at once as a medium for accumulation, transportation and demonstration of the antibodies. In plants there is no question of the production of the same type of immune bodies as in animals, but plants have no comparable medium for accumulation, transportation, and demonstration of such bodies. The result of such a situation would necessarily be that the antibodies in plants would be more strictly localized within the infected tissues and that demonstration of them would necessarily involve the use of the living cell. Thus far all of the experiments in the demonstration of plant antibodies which have given inconclusive or negative results have employed the techniques of either expressing the sap of plant parts or of macerating plant tissues in such solvents as water and physiological saline solution. In either case the cells are killed, substances discrete in nature have combined, and many substances have experienced irreversible change. This has been considered to be an application of animal serology to plants but it is far from that. Experiments on anaesthesia of plant cells and on the most gentle methods of killing plant cells have revealed that the immunity of the cells is lost not only on the death of the cells but even on their temporary narcosis. There is therefore no more reason for regarding the negative results of the "serological" tests in plants as evidence disproving the thesis of plant acquired immunity than there would be for expecting to obtain evidence as to the

cytology of the apple fruit from a microscopic examination of cider.

Acquired immunity in plants is a vital phenomenon. It is a function of the living cell and it must be studied in the living cell since plants have no such mechanism as animals for serological study. This is not an impossible requisite. Bernard has shown how the serology of the living orchid cell may be studied. Micrurgy is another point of attack. The artificial culture of living tissues in vitro may likewise afford an aid in this direction, and doubtless other techniques will be devised. Suffice it to say that there is abundant evidence thus far from the study of living plants and tissues that the acquirement of immunity to foreign bodies is a function not of animals alone but of life. In plants it is manifested chiefly or entirely in the reactions of living cells, which leads to a somewhat stricter localization of immunity than in animals. This implies that in plants one must attack the problem from the standpoint of the living cell. The techniques and the principles of the rôle of acquired immunity in plant life must both start from this basis.

It is not meant to be implied that there is no circulation of the antibodies in plants. Undoubtedly there is some such circulation, although it must be very limited as compared with that in animals. It may be that such circulation operates directly through the protoplasmic cell connections, and it is also not impossible that the circulating saps of the plant may serve to a limited extent as media for the transportation of antibodies. This latter possibility has been particularly investigated by Carbone and Arnaudi (45–47) but thus far without conclusive results. Be that as it may, there is assuredly no close analogy between such antibody circulation and that in animals and we are accordingly compelled to direct our attention in the main to the behavior of living immunized cells.

### *B. The rôle of acquired physiological immunity in phytopathology*

We have seen that acquired physiological immunity is exhibited in an important and widespread manner in parasitism and in that special branch of parasitism, symbiosis. In nature there is no question, from the findings reported above, that such immunity is a common and ubiquitous physiological property of plants. This being so it must exert an important influence upon the occurrence and severity of plant disease in nature. In what respects and to what extent does it influence phytopathology? Light is shed upon this question by the following considerations.

Most plants are immune to most parasites. In a great many cases this general immunity is due to normal properties of the plant, such as the impervious character of the superficial tissues or the unfavorable external or internal environment of the plant. In many cases, however, we have submitted evidence to show that the defense of plants against parasites which in nature do not succeed in the parasitism of such plants is due to an acquired defensive reaction set up in the host as a reaction to the stimulus of the invader. That such a mechanism is responsible for a considerable amount of the relative immunity in nature is seen from the frequency with which one is able to demonstrate in host tissues the checking and killing of the vegetative body of the parasite. To be sure acquired immunity is only one of the protective mechanisms of plants. It is a mechanism which reaches its highest development in suscepts related to the natural host, where normal defenses are less abundant, and where immunity is distinctly relative. But in the evolution of parasitism tentative attempts at increasing this host range must be continually being made by parasites, and these attempts would naturally be chiefly directed

toward suscepts closely related to the natural host. It is in just such cases that acquired physiological immunity is most evident, and it naturally follows that acquired immunity must play a significant part in the host restrictions of parasites.

Passing to a broader aspect of acquired immunity in parasitism it is of interest to study the relation of acquired immunity to the epidemic occurrence of diseases. A regular cycle of progress marks the bringing together of new host-parasite combinations. If the conditions are favorable for parasitism and spread of a given pathogen, the disease very rapidly increases after its introduction. There eventually occurs a period of the height of epidemic severity which may or may not prove fatal to the host taken as a whole. If the host is not eliminated, eventually this epidemic severity begins to decrease until it reaches a steady point with little or no variation beyond that due to environmental and local factors. We must distinguish here between epidemics due to facultative parasites and those due to obligate parasites. In the latter case some degree of immunity is indispensible to the perpetuation of the disease since only living hosts may serve as substrata for the parasite. We must also distinguish between the diseases of annual and those of perennial plants. In the perennial plants the duration of life is so great as to permit several or many successive attacks of the parasite, and accordingly the opportunity for acquired immunity to function is considerably greater than that in annuals. We see then that acquired immunity would be expected to exert its greatest action in the case of diseases of perennial plants, notably trees, and in diseases caused by obligate parasites, e.g. the powdery mildews, the rusts, and the Exoascales. Indeed the observations which have been made of acquired immunity in nature have centered about these types of disease.

The effect of acquired immunity in checking the severity of epidemic tree diseases has already been mentioned with respect to the *Oidium* disease of oak, the *Gymnosporangium* rust of red cedar, the *Endothia* canker of chestnut and the *Hemileia* rust of coffee. It is thus apparent that in such diseases in nature the successive infections of trees result in some degree of acquired immunity which is expressed by gradual tolerance of the parasite, alternation of periods of infection and freedom from infection, increased age of coppice growth of infected trees before succumbing, and decrease of severity of attacks in the four cases mentioned. We see, accordingly, that although acquired immunity may or may not play a major rôle in the normal freedom of plants from disease, it does play a leading part in the gradual checking of epidemics of plant disease, a rôle which combined with natural selection of resistant plants serves to save the parasitized species from extinction.

From the results of this analysis it is also evident that acquired immunity has had a part to play in the evolution of parasitism and symbiosis. The nature of the relationship of the orchid to its mycorrhizal symbiont and of the host to its obligate parasite are exceedingly delicate and are conditioned by a precise acquired immunity of such a nature as to permit the survival of both members of the association. Evolution of the symbiotic and parasitic habits respectively has been governed by evolution of this property of delicately adjusted partial immunity. We do not need to look far to find many cases of thwarted parasitism and symbiosis, the lack of the adjustment being due to a lack of the proper immune relationship between higher plant and fungus. In the rusts such thwarted attempts are seen in hypersensitivity, where the immunity is too weak, and in the small abortive lesions which have been shown above to

be due to a precocious and too great acquired immunity. Likewise in the orchid symbiosis cases have been recorded where the union is unsuccessful because of the immunity deficiency on the part of the host or excess virulence on the part of the fungus, leading to virulent parasitism, or the converse in which case germination fails to occur. Evolution of the parasitic and symbiotic habits in these two types of association, which assuredly represent the end results of long periods of adaptation, must thus necessarily have been concerned with the evolution of virulence on the part of the fungus and of the ability to control this virulence through the mechanism of acquired immunity on the part of the vascular plant.

### *C. Practical applications of acquired immunity in phytopathology*

In human medicine an application of the principles of acquired immunity has afforded some of the greatest practical aids in curing and preventing disease at the disposal of the physician. No such great practical advantage can be prophesied for an application of acquired immunity in plants, however, because of the inherent difference in the purposes of plant and human pathology. The aim of human medicine is to preserve the individual; the phytopathologist, on the other hand, as a rule has little interest in the individual, his main objective being the preservation of the population. Moreover while much of the activity of the physician is devoted to cure or therapy, that of the phytopathologist is more directly concerned with prevention or prophylaxis. Yet in spite of this it is not inconceivable that the principles of acquired immunity in plants may have practical applications in pathology and that the science may have not only theoretical but commercial importance. In the forest, the plantation, and the crop field it is doubtful that much in the way of biological therapy and prophylaxis can be accomplished. In ornamental plantings, in seed beds, and in the greenhouse, however, where the value of the individual plants is much greater, a serious consideration of the application of the findings of acquired immunity is warranted.

Little or nothing has been done in a practical way in turning to account the results of investigations in this field. Some have even objected to such an application on theoretical grounds, basing their argument upon the frequently temporary character of the immunity. Yet numerous vaccination experiments have proven that plants may be immunized and in some cases strongly so by such treatment. Leemann (102) has pointed out that the resistance and susceptibility of plants may be varied by the treatment of the soil with various biological products. In such diseases as damping-off of seedlings, potato tuber diseases, and even possibly in diseases of shrubs and trees where the individual plant is worthy of treatment, there is a field for experiment in the practical application of the principles of acquired immunity. Sieden and Trieschmann (158) have made a start in this direction and have been able to increase appreciably the potato yield as a result of a relatively simple tuber treatment.

One cannot go far in theorizing on the practical outcome of the knowledge thus far available, particularly in view of the almost total lack of experiments in practical application. Even were such practical applications not found to be worth while, the theoretical contribution of acquired immunity toward interpreting and understanding various pathological and medical problems justifies the study of the field, but assuredly we have here a line of attack for empirical pathological

problems which must needs be carefully sounded out.

*D. The direction of future activity in this field*

During the course of this paper numerous suggestions have been made as to desirable lines of future investigation in the field of acquired plant immunity. They are concerned with several main problems, namely:

1. Further confirmation of the *occurrence* of acquired immunity by filling in the several gaps which exist in the body of experimental data, by extending the results of the earlier investigations to a broader scope, and by strengthening by more carefully controlled experiments the less conclusive stages in the argument. Reference to the several sections of this paper will afford specific illustrations of this, and no attempt will be made to enumerate them all below because the weaknesses of each section will be best made apparent to the reader by his own perusal of the assembled data. Specific directions with regard to the procedure in certain types of experimentation have been given.
2. A study of the *nature* of acquired immunity in plants. Very little has been done in a critical way in this phase of the work. The evidence indicates that acquired immunity in plants is a vital, cellular phenomenon. Such methods as tissue culture and micrurgy are indicated from this fact. Large-celled plants such as certain of the algae, the coenocytic fungi, and the Charales should prove valuable material in this connection. Because of the essential similarity of acquired immunity in plants and animals and because of the relative ease of investigation of plants as compared with animals in certain respects, this type of investigation should be of interest to the animal serologist.
3. The study of the *theoretical bearing* of acquired immunity in pathology. The inclusion of the concepts of acquired immunity within the theoretical considerations of the plant pathologist is certainly indicated by the findings thus far. The pathologist cannot neglect in his analyses the importance of this phenomenon as has been seen from our brief survey of the significance of acquired immunity in plants as set forth in the preceding section.
4. An investigation of the *practical applications* of biological therapy and prophylaxis. There is practically a total lack of such investigations and the preceding subsection has pointed out concrete problems confronting the pathologist which should be attacked from this standpoint.

## X. RECAPITULATION

The present paper is a critical analysis of the problem of acquired physiological immunity in plants. It has been prepared in an endeavor to organize and clarify our knowledge in this important and difficult field of phytopathology by a determination of the occurrence and rôle in plants of defensive reactions of the zoöimmunitary type and of the theoretical and practical significance of such reactions.

In order to prepare the way for a clear understanding of the thesis, attention is given in an early part of the paper to the subject of immunological conceptions and terminology and to a short chronological account of the main steps in the develop-

ment of the concept of acquired immunity in plants.

On the basis of our knowledge of the factors conditioning acquired immunity in animals and of the physiology of plants, an analysis is made of the possibility of the occurrence of acquired immunity in plants as indicated by *a priori* deduction. The main *a priori* objections to such a thesis, i.e. differences between plants and animals with respect to circulatory system, manner of growth, opportunity for sensitization, and reaction toward disease, are in no case found to be valid objections, and on the other hand there are a certain number of facts which definitely lead to an *a priori* expectation of demonstrating acquired immunity in plants

In order to recognize and evaluate acquirement of antibody potency in plants it has been first necessary to investigate the presence and nature of normal plant antibodies. Such an investigation has revealed that antibodies of the zoöimmunitary type normally exist in many plants but that an identification of them is complicated by the frequent presence in plants of substances which exert actions simulating those of the true antibodies (pseudoantibodies). Thus both true agglutinins and pseudoagglutinins, true lysins and pseudolysins, and true precipitins as well as pseudoprecipitins have all been identified as normally occurring in plants. In addition to these there are present in certain plants growth-inhibiting substances which are probably of the pseudo type.

Acquired immunity in plants may be investigated by the artificial introduction into plants of foreign bodies, by a study of the reactions in parasitism, and by a study of behavior in symbiosis. The three subjects have accordingly been treated in turn with the following results:

Experiments in the introduction into plants of non-protein toxins have revealed that although the evidence regarding antitoxic effects in plants is very limited it seems probable that under some conditions at least plants can either acquire a tolerance to or actively combat foreign non-antigenic toxic material by reactions of the type of antitoxic reactions in animals. The artificial introduction of soluble proteins into plants has not been found by most investigators to lead to anaphylactic shock, although a very interesting phenomenon resembling this has been observed in sensitized bacteria. Beyond one observation of pseudoprecipitins no experiments have been performed to determine the acquirement of precipitins due to the injection of soluble proteins, an important phase of work which should be investigated. The evidence for the production of antibodies as a response to the introduction of particulate antigens is not wholly satisfactory because of the lack of completeness of most of the experiments, because of the action of unknown variables, and because of the presence of pseudoantibodies. The evidence is insufficient either to prove or to disprove the thesis under consideration but it is valuable in showing the dependence of acquired immune reactions upon the living state of the cell, in recognizing and allowing for the action of pseudoantibodies, and in directing future investigation in this field.

That immunity analogous at least to that of animal immunology may be acquired as a result of parasitism has been demonstrated by several types of investigation. In the first place the observations of many investigators are mutually confirmatory in finding that plants recovering from a first attack of a disease display a greater or less resistance to a second infection. This acquired immunity may in some cases be weak, temporary, or localized, and because of this and other difficulties in this type of investigation the need and requisites

for satisfactory demonstrations in this field are discussed in detail. Secondly, practically all of the numerous experiments thus far reported are in agreement in finding that the vaccination of plants, whether with attenuated strains of parasite, with extracts of parasite, or with antibodies of plant or animal origin, results in an increased and often very highly increased resistance of variable duration toward subsequent parasitic infection. Thirdly, the behavior of parasite and host when in contact has been found in many cases to be interpretable only in terms of an acquired immunity of the host toward the parasite. The same is true when one considers the complexity of the host specialization of certain parasites, notably the rusts, and the dependence of immunity upon a living, active condition of the host. Finally, the activity of plant hosts in restricting and killing their invading parasites is more satisfactorily interpreted by an hypothesis of acquired immunity of host toward parasite than by any other hypothesis yet advanced. In passing, the subject of hypersensitivity as displayed by the rust fungi is discussed in the light of its bearing upon the problem in hand. Serological demonstration of antibodies in parasitism has been almost universally unsuccessful, doubtless due to the difficulty of adapting serological techniques to plant materials and to the essentially vital nature of the substances responsible for the acquired defensive reactions observed in parasitism under the various conditions enumerated above.

Acquirement of immunity of the zoöimmunitary type in symbiosis has been thoroughly demonstrated in the behavior of orchids toward their mycorrhizal symbiont and of Leguminosae toward their root tubercle-bacilli, the demonstrations depending on both morphological and serological behavior. In addition analogous phenomena have been recognized in so many other types of symbiosis that the evidence strongly indicates a general occurrence of acquired immunity in symbioses throughout the whole of the plant kingdom. On the other hand, the graft-symbiosis has failed to prove satisfactory material for the study of acquired immunity in spite of the promise of early experiments.

On the basis of the experimental findings an analysis is made of the nature of acquired physiological immunity in plants. It has been shown that there is abundant evidence from the study of living plants and tissues that the acquirement of immunity to foreign bodies is a function not of animals alone but of life in both kingdoms. In plants it is manifested chiefly or entirely in the reactions of living cells, which leads to a somewhat stricter localization of immunity than in animals. This implies that the techniques and principles of the rôle of acquired immunity in plant life must start from this basis. Acquired immunity in plants has been found to play an important part in both plant disease and in symbiosis under natural conditions. It has important effects in limiting the host distribution of parasitic fungi, in checking the severity of plant disease epidemics, and in the evolution of highly specialized obligate parasitism and symbiosis.

The possibility of the practical application of the principles of acquired immunity in plants is discussed. It is found to be a desirable line of investigation, although little or nothing has yet been done in this field, and accordingly suggestions are made as to the practical advantage which may be taken of acquired immunity in plants.

Finally a number of suggestions have been made as to the direction of future activity in this field of study. The desirability of investigations regarding the occurrence, nature, theoretical bearing, and practical applications of acquired plant immunity are outlined and discussed.

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(Literature bearing on the theme of this review but received too late to be incorporated into the text.)

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# MY UNCLES, LOUIS BEDEL AND HENRI D'ORBIGNY

## BY MAURICE BEDEL

NOTE

This sketch of two distinguished French entomologists appeared in the centenary volume of the *Société Entomologique de France* (1932, pp. 95–99). The author, Maurice Bedel, has courteously given me permission to translate this tribute to his uncles. He is himself an entomologist, though better known as the author of three charming and humorous novels, "Philippine," "Monsieur Molinoff" and "Sulphu." W. M. WHEELER.

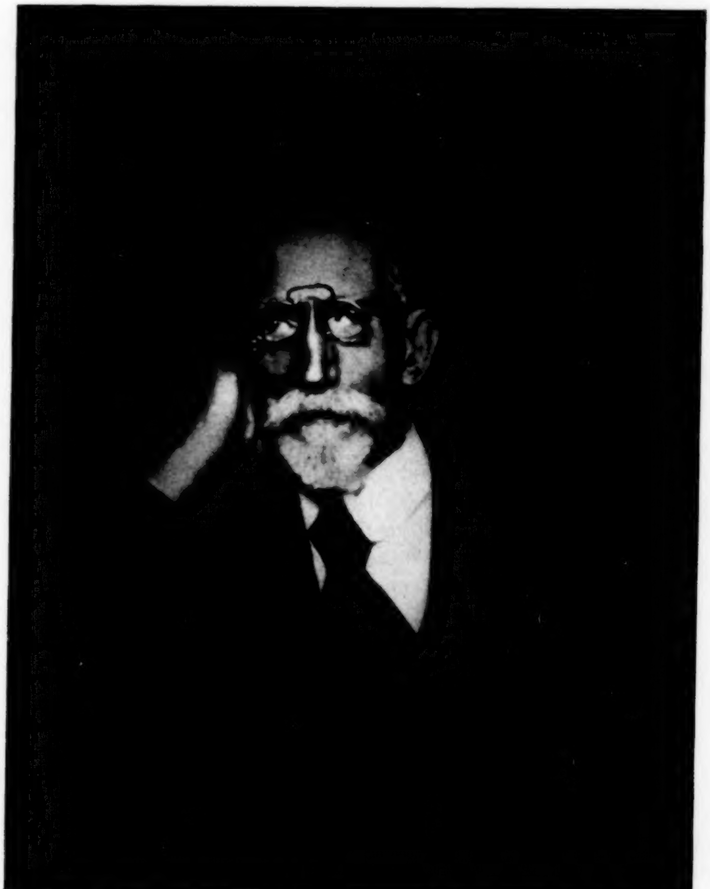
MY GRANDFATHER was a councillor of the court and a lepidopterist. As soon as he could doff his red robe, he seized his green butterfly net and ran about in the forest of Meudon, where, seventy-five years ago it was still possible to pursue butterflies without risk of falling into an ambuscade of boy scouts. My father and my uncle Louis accompanied him. My father, being an artist, delighted in contemplating the tiger-beetles along the sun-lit paths, while my uncle pursued the ravishing beasties and popped them into his bottle, because his youthful brain was already seething with the catalogues and synopses which were later to make him famous. Thus arose, between the two brothers, the differences which were only to increase during the course of their lives, till they lost each other from view forever.

My uncle remained a mystery to me till my father's death, when I was sixteen. He was described to me as a man devoid of sociability, preferring the tables of the cafés of the Boulevard Saint Michel to the family board, frequenting during the summer the inns of the Paris suburbs and there leading a celibate existence whose secret goings-on could not fail to scandalize his other brother, who was an abbé, and his sister, Madame d'Orbigny, who was virtuous.

Eventually I made his acquaintance. From the very first he seemed to me to be a man with whom only entomological conversation was possible. It was at a family luncheon, the first he had deigned to honor with his presence, at my uncle d'Orbigny's. Notwithstanding the excellence of the victuals and wines, and the presence of several ladies who were utter strangers to matters coleopterological, he started off, the moment the oysters were served, on an acrid and fiery criticism of his colleagues in the Entomological Society. Without any restraint he denounced the omission of a comma in a text with as much vehemence as the confusion of a species with a variety. I marvelled while he neglected the mushrooms and meat in the pâté in order to devour at his ease the honorable members of a learned Society. "Behold," said I to myself, "a man of knowledge who exalts science above friendship, above his family and even above good food. He certainly is somebody!"

Though I was greatly intimidated, I managed to screw up my courage to address him, expecting to rise in the estimation of so distinguished an uncle. I asked him about De Vries' theory of sudden mutations, which at that time had all the splendor of novelty. He glared penetratingly at me through his eye-glasses, snapped out: "Of no interest!" and continued to descant on I know not what error in Reitter's "Catalogue."

He was one of those censors of science, who, both formidable and actually feared, carry on without respite the combat against the negligence and ignorance of others. It must be admitted that the reliability and high excellence of his own works gave him a right to play this rôle. If he lacked general ideas, if the great problems of biology left him indifferent, he was none the less one of the founders of modern entomology. I leave to others more competent than myself the task of evaluating his contribution to science in the works he has bequeathed us.*



Louis Bedel (1849–1922)

* [An excellent necrology of Ernest-Marie-Louis Bedel by J. Sainte-Claire Deville is published in the ninety-first volume of the *Annales de la Société Entomologique de France* (1922, pp. 165–189). Lest some of

I wish only to speak of the man. He dwells in my memory as the type of that human species which we call the savant, a species now on the road to extinction and being replaced by the man of science, a new species represented in the bosom of the Entomological Society of France by a considerable number of specimens of excellent quality.

There are many descriptions of the savant. Every psychologist, novelist, moralist, comedy-writer, pamphleteer, fabulist, popular editor, song-writer or worldly conversationalist has evolved his own definition of this creature. Louis Bedel agreed very closely with the average type of their descriptions. If he failed to let his hair grow long, if he did not wear a redingote embroidered with the academic palms, and if he was not sufficiently absent-minded to mistake the back of a cab for a blackboard, he embodied, nevertheless, the tradition of the legendary savant in his dwelling and his mode of life.

The apartment which he occupied during the greater part of his existence, at 20 Rue de l'Odéon, was extraordinary. Not everybody who wished to, could enter it. You might pull at the bell-rope in vain; if you had not taken the pains to ask for an audience, the door remained closed. By a kind of diabolic intuition, my uncle seemed to divine the quality of the visitor from the sound of the bell and whether it announced an importunate bore or a friend. Even I, who in the early stages of our acquaintance, failed to take the precaution of announcing my visit beforehand, have many a time climbed the four flights of stairs to his domicile in vain.

If you were admitted you at once found yourself in a chaos of books and pamphlets,

our younger entomologists infer that this investigator's ideals are no longer worthy of emulation, I have translated a few of Deville's paragraphs.

"It is somewhat presumptuous to wish to characterize in a few words the tendencies and methods of an investigator like Bedel. Nevertheless, it seems to me that he may be said, not too inaccurately, to have possessed an essentially analytic and objective mind.

"Like most intelligences of this type, he instinctively applied the famous rules of Descartes, and God knows how rigorously! All of us remember how meticulously he mounted to the sources, to the original memoirs, and verified the most insignificant note and citation. He excelled as greatly in passing from the simple to the complex, from the known to the unknown as in subdividing difficulties for the purpose of solving them and of delimiting and evaluating their component parts, till they had lost their previous obscurity and were illumined throughout.

"So far as taxonomy is concerned, he possessed in its highest perfection a sense of the hierarchy of characters. His constant preoccupation was the discovery and elucidation of the organic and essential, though often concealed, as contrasted with the superficial and fluctuating, characters. He knew how to reject, deliberately, the details of no importance which are apt to burden descriptions. How many others, possessed of the desire to be exhaustive, have succeeded only in being at the same time both diffuse and confusing!

"Though above all a describer, Bedel never lost interest in biology. In the Phytophagous Coleoptera, especially, he was always careful to summarize their development in a brief formula (food-plant, portion of plant attacked, pupation in the earth or *in situ*, period of oviposition and eclosion, etc.). His own observations on these matters have enriched science with many a previously unknown fact. Finally, thanks to the critical sense, which he possessed in a high degree, he demolished a great many legends and doubtful assumptions.

"But the most precious of his natural gifts was certainly that of knowing how to express clear ideas clearly. He excelled in persuading his readers to participate in the lucidity of his analyses. His descriptions are quite devoid of the subjective element, which is so disturbing in certain authors. On reperusing their descriptions, these authors, because they can recognize their conceptions in an improvised and faulty text, have an illusion of clarity and feel no need of improvement. Bedel, on the contrary, puts himself in the reader's place and retouches his text till it becomes, so to speak, a faithful and concise image." W. M. WHEELER.]

plunged in the darkness of what must formerly have been a reception room. The window was blocked up with all kinds of bulletins, catalogues and offprints. On the right was the former dining room metamorphosed into a library specially reserved for the science and literature of travel. Unfortunately, access to the shelves was impossible, owing to the intervening mountains of volumes, so that in order to reach Asia it was necessary first to demolish Africa. Advancing somewhat farther you came to the kitchen, where for thirty years no chicken had been browned nor any meat stew prepared. This room, too, contributed its full share to the city of books. The stove, the sink and the pantry were hidden under the *Voyage de Pallas*, the *Voyage de Bruce* and the *Collection Choisie de Voyages autour du Monde*—never had the explorers of two hemispheres felt themselves confined to such cramped quarters.

On the left the visitor discovered among other shelves groaning under the weight of entomological annals, bulletins, revues, miscellanies and catalogues, the collections of Louis Bedel themselves, housed in vast glass cases and perched on many chairs. We are now in the drawing room and it seems natural that this place of honor should be reserved for the guests of quality that streamed to the Rue de l'Odéon —I mean the Coleoptera, many of which were great personages—the "types." It was therefore impossible to find a seat, the chairs, lounges and even the humblest stool being reserved for the cartons and boxes. Passing now into the bed-room, you observed that my uncle shared it with the Chrysomelidae, the Staphylinidae, the Harpali and the Catops—all of which, since my uncle was a bachelor, was most edifying. I will add that the bed was covered with clothing and hats because the closets were also reserved for the boxes, that the writing table was swamped beneath entomological materials and printed matter of all sorts, and that my uncle, seated on the extreme edge of a chair piled with the latest numbers of the *Annales* and *Bulletins* of the Entomological Society of France, was often obliged to use his knees as a desk for the composition of a large number of his works. The only human note in this capharnaum emanated from a painting representing my grandfather at the age of fifteen—all the rest was bottles of cyanide of potassium, boxes of pins, slabs of cork, pocket-lenses, pots of glue, tweezers and labels.

Such was the setting. The man who dwelt within it was not, as I have had occasion to remark, easy to approach. He shrank from new-comers; he bristled up in the presence of phrase-makers; he growled his disapproval of the ignorant. And he had justification, for science is bound to defend itself against parasites and commensals. It was, I am sure, one of the great merits of this general honorary secretary of the Entomological Society to track down and destroy amateurishness in a department of science in which it is only too apt to establish itself.

From my tenderest years and owing to the beneficent star under which I was born, I have always loved entomology, but as I was only an amateur in this department of the natural sciences, my uncle treated me with scant affection. I could not make him appreciate the fact that the insect was leading me directly to man, and that a novelist cannot venture into the thicket of human phenomena till he has explored the infinitely more mysterious and therefore more fascinating world of animals and plants.

His brother-in-law, my uncle Henri d'Orbigny, was more naïve in his reasoning and much more urbane in his manners. He was descended, as we know, from an illustrious family, and celebrated entomology

as a religious rite; it was with him a constant obsession. Occupied with the study of the dung-beetles (Coprophagidae) and especially with that of the African Onthophagidae, of which he described several hundred new species, he worked incessantly except during three months of the year. Then he hurried to some place in the Alps, where he hiked with a kind of fanaticism, climbing hostile slopes and refractory summits till his seventieth year. He then botanized with great ardor and would have broken his neck to reach some rare species of *Gnaphalium*. This was his only infidelity to the Onthophagi.

I often accompanied him on these summer excursions. At that time I was very young and have retained an excellent recollection of my white-haired companion, whose enthusiasm and youthful impulses accorded so well with my own. He was in an ecstasy of delight at the sight of a sulphur anemone; the discovery of a rare Crassulaceous plant would make him shout till the mountains resounded with the echo of his voice. For my part, I pursued the insects, I swept and beat the vegetation and filled my bottle. On returning to the hotel I studied the collected game with my uncle. It was fascinating. Never have I known joy of just that quality during my subsequent researches on the human fauna of the European capitals. My uncle must have had a presentiment of my fate, for he could not invent enough sarcastic remarks on literature and all literary men, with the single exception of the great writer Maurice Maindron, who was forgiven much because he was also an entomologist.

At Paris it was arranged that I should dine with my uncle once a week. My aunt, who was of a talkative disposition, was permitted to entertain us during the meal with the futilities of mundane life, but as soon as the dessert and the fruit had been swallowed, we repaired to the laboratory, and it was also the rule that I should start the conversation on the new species of *Onthophagus* that had been described during the week, for my good uncle's whole life had been regulated, ordered, classified, catalogued and reduced to synoptic arrangement—his relations with his friends and family, his admirations and sympathies, his nourishment and the employment of his time.

Passionately he described the prothoracic punctuation of *Onthophagus sexcornutus* d'Orb. as "simple, fine or rather fine and rather sparse;" then that of *O. multicornis* d'Orb. as "simple, rather coarse and not dense." I could see no difference, and told him so, but he was pained, because he was proud of the minuteness and accuracy of his descriptions. Then we passed to the frontal carinae, which in the one species were "more or less short;" in the other "rather short."

"That is six of one and half a dozen of the other," said I.

"My friend," said he, "there is a shade of difference that eludes you."

I was always accused of not understanding the French language, which is extremely difficult, I admit, but I persistently denied any scientific value to such vague terms of comparison as "interstriæ provided with rather dense granules" and "interstriæ with somewhat separated granules," and this would throw my uncle into violent fits of bad humor. At bottom it really pleased him, because, though apparently very peaceful, he loved to quarrel, especially about statements. In this respect he differed from his brother-in-law, Louis Bedel, who quarreled only about men. The two brothers-in-law therefore never understood each other.

"Have you read," asked d'Orbigny, "the latest communication of X?"

"Absurd!" replied Louis Bedel.

"Why?"

"Because it is X's."

On another occasion, it was Louis Bedel, who asked:

"What do you think of Z?"

"I deny him all authority!" replied d'Orbigny.

"Why?"

"Because he is the author of such a work."

Such were my two uncles. Owing to a mental idiosyncrasy that was later to cause me much annoyance in my career as a novelist, I saw them only from the distorting angle of ironic observation. This will be evident from the perusal of the few lines which I have consecrated to them. I honestly deplore this manner of celebrating the memory of relations who were dear to me, but what am I to do? An honest writer should remain himself, especially in an apologia for members of his own family.

# THE EVIDENCE FOR INHERITANCE OF RESISTANCE TO BACTERIAL DISEASES IN ANIMALS

By W. V. LAMBERT

(*Paper No. 55 from the Department of Genetics, Iowa State College, Ames, Iowa*)

THE COÖPERATION of two sets of circumstances is necessary for the development of an infectious disease. In the first place, the organism in which the morbid process is to develop must conform to certain conditions of structure and function, while secondly, an external agent, the bacterium in the case of bacterial diseases, must exert a specific effect upon the organism which is in the process of developing the disease. These facts have long been a commonplace in medical writings (see Bulloch and Greenwood (1911) for a review of the older literature), but unfortunately little attempt has been made to evaluate the rôle played by the host in disease processes. Because of the obvious need for combatting disease through control of the infective agent, most effort, naturally, has been directed toward a study of the causative agent and to the series of changes that follow its invasion of the tissues of the host. Nevertheless, the fact remains that the constitution of the host plays an important part in disease resistance, and it is probable that a more exact evaluation of this rôle will be of fundamental importance for students of epidemiology.

Much statistical and observational evidence on this problem exists, but for the most part it allows only for the general conclusion that marked differences in disease resistance do occur among animals. Attention need hardly be called to the fact that the diseases of one species are generally not pathogenic for the individuals of other species, or if so usually only to a mild degree. In many cases this resistance is so great as to be spoken of as a natural immunity.

Intra-species differences in resistance likewise exist. It is a commonly observed fact that some individuals survive severe epidemics without apparently contracting the disease, others are affected but slightly, whereas some succumb to the ravages of the disease. Many of these differences can be explained, no doubt, by environmental causes but it would be rather presumptuous to assume that all of them are due to accidents of environment.

In man, many well-founded cases of racial differences in resistance to infections have been reported. Thus, it has been pointed out by Ferguson (1928) and others, that tuberculosis is far more fatal to the peoples of uncivilized races than it is for the people of regions in which the disease has been endemic. Racial differences occur, however, in regions where tuberculosis long has been endemic. For instance, Dublin and Baker (1920) found a significantly higher death rate among the native born Irish of Pennsylvania than among the native born Italians. The difference was greater than could reasonably be explained by habits of life, differences in social or economic status or by environmental causes.

For diphtheria also racial differences in resistance occur. Holmes (1931) has pointed out that the negro is more resistant than the white man. In this case

Holmes suggested that the greater resistance might be due to a thicker ectoderm. For pneumonia likewise racial and even familial differences in resistance have been noted. Thus, Pearl (1926) has reported one family of 13 in which the incidence of pneumonia was 100 per cent, a condition which he concludes could have been in no significant degree environmental. The evidence in this case indicated that the whole family possessed a definite constitutional inferiority of the respiratory system.

In animals numerous instances of subspecific and breed differences have been observed. Tyzzer (1917) and Hagedoorn-LaBrand and Hagedoorn (1920), for example, observed marked differences in resistance among mouse stocks in their laboratories. The ordinary mouse (*Mus musculus*) was far more resistant to the infections than was the Japanese waltzing mouse (*Mus bactrianus*).

The most complete evidence of strain differences in disease resistance has been reported by Wright and Lewis (1921). In their experiments guinea pigs from five highly inbred lines were infected with tuberculosis and marked differences in resistance were found between some of these families. The high resistance of one family was transmitted alike by males and females when crossed with one of the other inbred families. In the latter crosses the $F_1$ progeny were superior to the resistant parent family, thus indicating the presence of complementary factors for resistance. Over 30 per cent of the variation in length of life among the crossbreds could be attributed to the amount of blood of the best inbred family.

Much other evidence of this sort might be cited but a large part of it has been reviewed by Crew (1928), by Kozelka (1929), and for man, by Holmes (1931). More recently, Darling (1932) has reviewed some of the literature and has emphasized the inadequacy and uncritical nature of much that has been written on this subject.

THE EFFECT OF SELECTIVE BREEDING ON HOST RESISTANCE

More critical evidence of the part played by the host on resistance to disease has been advanced in the last decade from laboratory experiments undertaken primarily to study this question. In most of these studies such factors as the degree of infection, route of infection, pathogenicity of the bacterium and the environment were controlled, as well as such factors as age and condition of the experimental animals. In addition, the cause of death in most instances was reasonably well established.

The investigations of Webster (1924 and 1925) were among the first to demonstrate the influence of selective breeding on host resistance. By the simple process of using only survivors of a mouse typhoid infection Webster was able to bring about a marked decrease of mortality from this disease in the course of a few generations. On the other hand, he advanced data to show that the offspring of females most susceptible to this infection produce, in turn, offspring more susceptible than a similar group of unselected mice. While the experimental data were too few to allow for sweeping conclusions, Webster's experiments had the merit of being carried on in such a manner as to reduce to a minimum the possibility of a specific acquired immunity.

A more critical investigation of the genetic basis for resistance to mouse typhoid was conducted by Schott (1932). In his experiments the breeding mice not only were survivors of a standard infection with the causative bacterium, but they came from those families showing the highest progeny resistance. As a result of

six generations of such selective breeding the mortality in the selected stock was reduced to 24 per cent, whereas the foundation stock from which the selected population descended showed a consistent mortality of over 80 per cent. A control (unselected) population, consisting of 538 animals of the same strain as the selected stock, that were tested at intervals throughout the six generations, showed 82 per cent mortality. Rate of mortality as well as total mortality was decreased by selection. The matings in this experiment were so directed as to concentrate the blood of those individuals giving the highest progeny resistance, and it was found that a large part of the high resistance of the selected population could be traced to the influence of a few "key" animals.

Hetzer (Unpublished data) has continued this investigation and finds that selection is still effective within this stock, but the increase in resistance has been at a rather slow rate, a situation that also was observed by Schott in the later generations. It is probable that the end point of selection in this stock has nearly been reached, for the degree of inbreeding within the selected stock is now very high. Hetzer has increased the standard infection fourfold in the last generation with a resultant increase of approximately 9 per cent in mortality. This fact would indicate that there is a rather delicate balance between those innate factors for resistance and such environmental factors as degree of infection.

Using a similar plan of selection Irwin (1929) was able to increase the resistance of the rat to a standard infection with the Danysz bacillus (*Salmonella enteritidis*). Three generations of selection resulted in a decrease in mortality from 85 to 35 per cent. In this experiment, as in those of Webster and Schott, the greatest effect of selection was observed in the first generation.

Similar experiments conducted in chickens by Roberts and Card (1926) using *pullorum* infection and by Lambert and Knox (1928) for resistance to fowl typhoid have given quite similar results to those reported above. Recently, Roberts (1932) stated that the strain of fowls selected for resistance to *pullorum* infection was much more resistant than fowls of the unselected population that were so infected, the respective mortalities being approximately 35 and 73 per cent. These differences have been reasonably consistent for seven consecutive generations. His investigations, furthermore, have shown that chicks from some matings are far more resistant than those from other matings and that these differences are very consistent. Lambert (1932a) has been able to reduce the mortality from fowl typhoid from 85 per cent to 10 per cent as a result of five generations of selective breeding. Since much variability was observed for different matings in the resistant stock in the latter experiment, even after five generations of rigid selective breeding, it would appear that considerable genetic variability still existed in this stock.

Recently, Manresa (1932) has shown that marked inherent differences in resistance exist in rabbits to infection with *Brucella abortus*. He was able to differentiate rabbits into reasonably true breeding susceptible and resistant lines by a program of careful breeding based upon the female's reaction to an injection of virulent *B. abortus* bacteria. In this study the criterion of resistance was the ability of a doe to bring to full term a litter of normal young rabbits following an intraperitoneal injection of the bacteria near the fourteenth day of pregnancy.

Frateur (1924) proposed a mono-factorial difference as controlling resistance

and susceptibility to fowl diphtheria, but his data are so few that this conclusion can be considered as no more than suggestive. Topley (1926) as a result of his extensive studies on the epidemiology of mouse typhoid clearly recognizes the importance of the hereditary element in disease resistance, for he states that a colony may increase its average resistance by a process of simple selection; namely, by the elimination through death of the more susceptible members.

### CAN A PASSIVE TRANSFER OF IMMUNITY BE PRIMARILY RESPONSIBLE FOR THE INCREASED RESISTANCE IN THE SELECTED STOCKS?

The experiments just cited prove that selective breeding is decidedly effective in increasing resistance to a given disease but it may be suggested that other factors could be responsible for some of this increased resistance, such as a passive transfer of resistance from the dam to her progeny. If this were true, then only a part of the increased resistance could be genetic in origin. While the continued effectiveness of selection over a number of generations would seem to refute this possibility, experimental data are at hand which show that a passive transfer of immunity cannot have played a major rôle in the increased resistance of the selected stocks.

In order to test the possible influence of this factor upon the resistance of selected offspring Schott (1932), Lambert and Knox (1929) and others early in their experiments made two series of matings to test this question. One series consisted of selected males by unselected females while the second series consisted of the reciprocal matings. The data from these experiments indicate that the male transmits resistance to the progeny in nearly equal degree with the female. Since Smith (1907) and others have shown that passive immunity is not transmitted through the sire it would appear that a passive transfer of immunity cannot have been responsible for the increased resistance of the selected stock.

Further evidence on this question has been advanced by Roberts and Card (1926) and by Lambert (1932b), who have shown that the progeny from surviving carrier hens are no more resistant than chicks from non-carrier hens. Again, the fact observed in their experiments that some pairs of birds produce far more resistant offspring than do other pairs of birds provides additional negative evidence against a major influence of the passive transfer of immunity.

Regarding the development of an active immunity previous to the time of receiving the test dose, the evidence is again largely negative, though in some cases this factor may have played a part in enhancing the resistance of the selected stock. Had it been a major factor, however, it would have been expected that the effectiveness of selection would have been reached in the first or second generations. As previously shown, this was not true. In the case of the experiments of Roberts and Card (1926) this criticism cannot be made for the chicks were inoculated when one day old and hence could not possibly have acquired such an active immunity. While it is true that some eggs produced by carrier hens do transmit the infection, the proportion of such eggs on the whole is small (see Weaver and Weldin, 1931), and thus in no sense could this factor have had a major part in bringing about a greater resistance of the selected stocks in these experiments. The experiments of Lambert (1932) furnish additional negative evidence of this sort.

The experiments of Webster, as previously mentioned, were conducted to reduce the possibility of such preinoculation infection to a minimum, so it could

not have been an important factor in his experiments. Manresa found that females produced by actively immunized susceptible does were no more resistant than were the females produced from non-immunized susceptible does. Furthermore his experiments were so conducted as to rule out either the passive transfer of immunity or an active immunity acquired by sublethal infection previous to the time all females were submitted to test.

The most convincing evidence on this point has been obtained by Gowen and Schott (unpublished data kindly furnished to the writer). Susceptible females of an inbred strain of mice were double mated to susceptible males of their own strain and to resistant males. The matings were made so that the offspring from the different males could be determined from the color of the offspring. From the susceptible males 20 offspring were produced all of which died when submitted to the standard infection of mouse typhoid bacteria. From the resistant males 15 offspring were produced and tested. Of this group only 8 died. While the data are few, this experiment clearly demonstrates the importance of genetic factors for resistance and shows, furthermore, that they are transmitted by the sire to his progeny.

In the face of all of this negative evidence it would seem justifiable to assume that a passive transfer of immunity, or the acquisition of an active immunity in the young stock, acquired by sublethal infection before test, have not played a major, if any, rôle in the increased resistance of the selected stocks used in the above experiments.

### THE GENETIC NATURE OF RESISTANCE AND SUSCEPTIBILITY

A genetic analysis of resistance and susceptibility to disease in animals presents many difficulties not encountered in the analysis of other characters. In the first place it is very difficult in most cases to establish clear-cut classes of resistant and susceptible animals. While the classification of dead or alive may be used, obviously such a classification does not take into account the various sublethal infections in the surviving population. Secondly, such a character as disease resistance is influenced markedly by environmental factors, some of which are very difficult to control. Finally, since disease in the sense used in this paper is a manifestation of the growth of an infectious agent in the tissues of the host, a variable is introduced which is not encountered in most genetic analyses. Obviously, therefore, it is mandatory in such studies that all external conditions, as virulence of the bacterium, degree of infection, and environment, must be controlled insofar as control is possible.

In spite of these obstacles, however, some facts have been presented that bear on this problem. From the mortality observed in stocks of ordinary mice, in Japanese waltzing mice and in their $F_1$ and $F_2$ hybrids, Hagedoorn-LaBrand and Hagedoorn (1920) suggested that the inheritance of resistance and susceptibility probably depended upon a monogenic difference. In this investigation the pathogen was not determined, nor was the degree of infection controlled, the mortality resulting from a spontaneous epidemic in their colony.

Frateur (1924), as previously mentioned, proposed a monogenic difference between resistance and susceptibility to fowl diphtheria, but the data are too few to be more than suggestive that this simple explanation is correct. Rosling (1929), from the results of reaction to the Schick test for diphtheria in 97 Copenhagen families, suggested that the rate of antitoxin production is governed by one pair of genes, individuals of the recessive type having

only a limited ability to produce antitoxin. The data of the Hirszfelds (1927) would not agree with such a simple interpretation.

Irwin (1929) crossed susceptible rats of an inbred strain with the first selected generation animals of a selected strain resistant to the Danysz bacillus. The $F_1$ individuals possessed an intermediate degree of resistance. $F_1$ individuals were crossed inter se and also back to animals of the susceptible strain. In the backcross generation stock about 50 per cent mortality occurred, whereas only 25 per cent mortality was observed in the $F_2$ generation. While these results might suggest a monofactorial difference between resistance and susceptibility, Irwin concluded that resistance was due to a complex of hereditary factors some of which are partially dominant. Similar conclusions were reached by Schott (1932) in regard to resistance to mouse typhoid and by Lambert (1932a) for fowl typhoid.

Roberts (1932) crossed chickens of a selected *pullorum* resistant strain with unselected birds, then crossed the $F_1$ birds to both the resistant $P_1$ and the unselected $P_1$. The $F_1$ generation was about as resistant as the $P_1$ resistant strain, showing only about 35 per cent mortality; the backcross generation from the cross of $F_1$ birds to the resistant $P_1$ showed a mortality of 37 per cent while the offspring from the $F_1$ by unselected $P_1$ gave a mortality of 62 per cent. In addition, $F_1$ birds were mated inter se. The $F_2$ progeny from these crosses showed a range from low to high resistance. Selections of breeding stock were made from the classes showing both low and high resistance in the $F_2$ generation. The offspring from the high segregates that were tested in 1931 showed a mortality of 31 per cent, while the mortality in the chicks from the low segregates gave a mortality of 92 per cent. These results show clearly the importance of genetic factors for resistance but do not permit of an exact estimate of the number of factors concerned.

Manresa (1932) tentatively concluded that the inheritance of resistance and susceptibility to infectious abortion in rabbits is governed by one pair of Mendelian genes and that resistance is dominant or partially dominant. Some objections were encountered to such a simple interpretation, however, and it is probable that more extensive tests will show the genetic basis of resistance and susceptibility in this case to be more complex than a monogenic difference.

In plants the genetic analysis of resistance and susceptibility to disease has been carried much farther than in animals (see Hayes, 1930, and others). Where a genetic analysis has been made in plants it has been mostly in self-fertilized species or in strains where, by long self-fertilization, a high degree of genetic purity or homozygosity has been attained. The use of such homozygous resistant and susceptible strains has made possible a clean-cut genetic analysis of disease resistance in some cases, as well as having furnished critical evidence on the physiological interrelations between host and pathogen.

In animals few highly inbred strains exist, and in those that do exist it is improbable that highly resistant lines will be found, since no selection for resistance was practiced during the development of such strains. Because of the complexities introduced by environmental causes and the interrelations of host and pathogen, it would seem that a careful genetic analysis of resistance and susceptibility to any disease must await the development of inbred lines which will react in a uniform and constant manner to infection with a given pathogen. Much progress, of course, may be made in the development of resistant strains without a genetic analysis, but a complete knowledge of the

part played by the host in disease processes will probably await a genetic analysis.

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# ON THE GENETIC STRUCTURE OF INHERITED CONSTITUTION FOR DISEASE RESISTANCE

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IT IS a familiar fact that even after repeated exposure to a disease there are some animals which will show no history of having had this disease. These refractory animals are, in common parlance, said to have a good constitution. In the literature on animal diseases it is frequently asserted that this good constitution for one disease is also apt to presage like resistance to another disease or unfavorable lethal entity of a quite different nature. In other words constitution is held to be a single unit type of character which enables the animal to resist a multiplicity of environmental agents. This concept has been built up largely around observation on animal diseases.

This view is opposed by the extensive experiments and observations which have been made on disease resistance in plants. These studies have almost uniformly led to the conclusion that genetic constitution for disease is a composite made up of numerous genetic factors, some making for resistance to one disease and some to another, the fortuitous combination of these factors leading to resistance to both.

The study of genetic factors which cause obvious pathological constitutional defects or death is also opposed to this view. A striking illustration of this fact is the case of a lethal factor which causes melanotic lesions of the leg joints in *Drosophila*. These lesions incapacitate the fly, causing its death. The inherited predisposition to this condition is specific, being dependent on a single inherited gene. This gene is entirely distinct from the 350 other genes which produced the other constitutional changes studied in the same experiments (1). Another equally striking case is that described by Mohr and Wriedt (2) in the Swedish breed of Holstein-Friesian cattle. In the homozygous condition one of these lethals produces congenital hairlessness accompanied with death, while the other causes the amputation of all prominent parts. The lethal factors causing these defects in the animal's constitution are widely distributed within this breed, due to the fact that two very prominent imported sires of excellent individual appearance were heterozygous for one of the pathological conditions, one for hairlessness, and the other for amputated extremities.

It is obvious that a marked and fundamental difference of interpretation exists between those who have studied animal diseases of bacterial origin and those who have studied plant disease of like origin or those studying pathology of either plant or animal of genetic inception. The question arises how are these two views to be harmonized.

The words specific and non-specific factors for disease resistance have been frequently used in discussion of this subject. These words have unfortunately different meanings in the two overlapping

sciences into which disease resistance studies naturally fall. In the immunological sense specific factors for disease resistance are taken to mean factors which cause resistance to one and only one disease, however closely related to it a second disease may be. In a still more restricted sense specific factors are sometimes thought of as identical with the immune bodies which the animal body forms after an attack of the disease and which are known to be quite specific for the given bacterial agent causing it. In this sense non-specific factors are defined as attributes which cause resistance to many agents. Genetic terminology of long standing has given these terms a quite different meaning. Specific factors have long been defined and thought of in terms of genes carried in the germ plasm. They are the inherited entities which guide body development to the formation of special characters. One of these factors may make for resistance to one or several lethal agents. Since this resistance is dependent upon one factor this factor is regarded as specific for it even though the developmental expression of it may have multiple effects. Non-specific factors are regarded more as variables of the environment, temperature, food available to the animal, etc. In this paper the genetic usage will be followed.

Genetic constitution could conceivably be either a single character which enabled the organism to resist a multiplicity of environmental agents or a composite of numerous independent characters each of which is capable of causing the individual to resist one environmental agent, the fortuitous combination of these separate characters making animals resistant to many or few such agents. The implications which follow in the wake of these two hypotheses are markedly different. If constitution were a single character its inheritance would be expected to be simple. If on the other hand it were a composite of many individual characters then its inheritance need not be simple. The consequences of epidemic disease and their bearing on future epidemic diseases within a herd composed of individuals made up according to the first hypothetical constitution are very different from those in a herd composed of individuals with the second hypothetical constitution.

### RESISTANCE IN MICE

The study of the genetic structure of constitution may perhaps best be approached by differentiating relatively pure and distinct lines within a species by matings designed to separate genetically distinct lines and then studying these groups exhaustively from the viewpoint of their reaction to agents chosen because they are believed to be distinctly different. For some years the work of our laboratory has been directed toward this problem. Among the genetic strains which we have collected and studied in these experiments were two which proved to be differentiated in their disease reactions. Throughout this work the writer has been associated with Dr. Ralph G. Schott.

The two lines S and sil were shown in the previous experiments to be distinct in their reactions to the pseudorabies virus. The S strain in a population of 190 showed the low survival rate of only $8.4 \pm 1.4$ per cent to this disease. The sil strain in the same experiment had a much higher survival, this rate being $52.2 \pm 2.5$ per cent in a population of 176. The $F_1$ hybrids' rate of survival was $23.7 \pm 3.7$ per cent in a population of 59. Many of those surviving the inoculation of the pseudorabies showed no symptoms of the disease, except that they became immune to a subsequent inoculation of a much larger

amount of the virus which would otherwise have been surely lethal.

On the basis of survival, the sil stock would be considered much superior to the S stock. If constitution is a general over-all single character in inheritance the sil stock would be expected to show a higher survival value for any other pathological agent. If on the other hand constitution is made up like other general somatic characters, composed of many separately inherited entities, the sil stock would not be expected to show higher survival value for other pathological agents.

TABLE 1

| | CLASSES COMPARED | $\chi^2$ | $P$ |
|---|---|---|---|
| Pseudorabies | S with sil | 84.5 | <0.001 |
| | S with $F_1$ (S × sil) | 10.0 | 0.002 |
| | sil with $F_1$ (S × sil) | 14.5 | <0.001 |
| Pseudorabies rates compared with *Salmonella aertrycke* rates | | 225.6 | <0.001 |
| *Salmonella aertrycke* | S with sil | 30.5 | <0.001 |
| | S with $F_1$ (S × sil) | 38.5 | <0.001 |
| | sil with $F_1$ (S × sil) | 110.8 | <0.001 |

It is possible to test this question since these two lines had previously been differentiated on the basis of their resistance to mouse typhoid, *Salmonella aertrycke* by his colleague R. G. Schott, Genetics 17: 203. The S line when inoculated with a standard dose of $5 \times 10^4$ organisms had 75.3 per cent of its individuals survive. The sil strain on the other hand had no mouse survive this dose. Crosses between these lines showed that the resistance of the S line was transmitted through both males and females. The survival rate of the $F_1$ crosses was 62.6 per cent. The typhoid disease behaved differently from the pseudorabies disease in the fact that all animals inoculated showed distinct symptoms of the disease. None escaped showing some traces of its effect. A test through the use of double matings has shown these differences to be genetic in origin.

The significance of these differences may be tested by their $\chi^2$ values. In the pseudorabies experiments there were 190 mice of the S line tested, 174 of which died and 16 survived. In the same experiment the sil strain had 176 mice tested, 84 of which died and 92 survived. The $F_1$ cross between the S and sil lines had 59 progeny of which 45 died in test and 14 survived. In the previous generations the test for the survival value of these three groups to an inoculation of a standard dose of *Salmonella aertrycke* organisms showed that of 105 tested in the S line 26 died and 79 lived; of 108 individuals tested in the sil line all died; and of 187 $F_1$'s tested 70 died and 117 survived. The $\chi^2$ and $P$ values for the different combinations are shown in table 1.

The survivorship curves shown in figure 1 bear out the same interpretation. The solid line represents the per cent of the sil strain surviving plotted against days after inoculation. The dot and dash line shows the same information for the S strain. The $F_1$ hybrids survivorship curves

are represented by the dotted lines. The plots are made on the same logarithmic scale so that equal rates are visible to the eye. The pseudorabies graph is quite distinct from that of the mouse typhoid in a number of particulars. There is first a definite incubation period. When deaths commence on the third day the survival line falls in very nearly straight lines from the initial origin of 100 per cent living animals throughout the cycle of the disease, when deaths cease nearly as sharply as they began. The cycle of the disease consists of only 3 day incubation period and 6 days when deaths are taking place. It will be noted that the S animals die at a much faster rate than the sil strain. The $F_1$ rate is approximately intermediate between the two parental strains.

The mouse typhoid curves present a markedly different picture. Deaths commence on the second day with a slow but constantly increasing rate. The sil and the S strain have now reversed their rates of death from those seen in the pseudorabies disease. All of the sil animals die off rapidly, the survivorship curve reaching zero on the fourteenth day. The S strain shows a slow rate of death and a high final survival value. The $F_1$ hybrids show somewhat less survival than the S parents and a markedly greater survival than the sil stock. The duration of the disease is distinctly longer with the mouse typhoid than with the pseudorabies with all strains.

These data show that the two lines of mice, S and sil, are distinctly different in their reactions to pseudorabies virus on the one hand and mouse typhoid organism, *Salmonella aertrycke*, on the other. The fundamental basis of these differences has been shown to be the genetic constitutions of these two strains. A genetic complex which is favorable to the survival of a strain when exposed to one pathological agent is here shown to favor susceptibility to another agent. The hybrids react like the S parents, showing a marked difference in dominance of the genes comprising the constitutions of these two lines. The genetic constitution for resistance or susceptibility to these diseases is shown by the data to be a composite of separate genes each capable of causing the individual to


Fig. 1. Survivorship Curves of the S and sil Strain and Their $F_1$ Hybrids for Pseudorabies and Mouse Typhoid

resist one environmental agent. The fortuitous combination of these separate factors makes animals resistant to many or few such agents.

These two lines of mice are simply two strains which have been differentiated genetically by breeding. A random bred stock would very probably contain both types well mixed within it. In point of fact in obtaining stocks from three different dealers in mice we obtained results

which would be called for by such a mixture. They exhibited the full range of variability which was found in the combined results from our resistant and susceptible lines.

The significance of these results to our problem is quite obvious. So far as this population, composed of these two strains, is concerned its resistance to these two diseases is on a distinctly different genetic basis. Selection for resistance to one of these diseases within this herd would result in susceptibility, not resistance, to the other. A constitution favoring resistance to both of these diseases simultaneously would be a fortuitous combination of the genetic resistances of both strains unless it were true that the genetic factors for resistance to one agent definitely produced susceptibility to the other.

#### RESISTANCE IN OTHER ANIMALS

Other work in the animal field has in part been interpreted as supporting this view of the composite nature of genetic constitution and in part that constitution was a single character. Lambert (3) has established a strain of White Leghorns which are resistant to the fowl typhoid bacterium, *Salmonella gallinarum*. Roberts (4) has established a strain of white Plymouth Rocks resistant to white diarrhea, *Salmonella pullorum*. Lambert (5) made tests of both strains for their cross resistance. Ninety-seven of the white leghorn strain resistant to fowl typhoid were tested for their resistance to *Salmonella pullorum*. Fifty-five per cent of the birds died on this test whereas for a like number of controls 86 per cent died. The generation of selection from which these resistant birds came had a death rate of slightly more than 15 per cent when inoculated with *Salmonella gallinarum*. Fifty-five of the White Plymouth Rocks resistant to *Salmonella pullorum* were inoculated with a standard dose of *Salmonella gallinarum*, with the result that 78 per cent died. The control group of 48 birds showed a death rate of 83 per cent.

The results for the birds which were initially selected for resistance to *Salmonella gallinarum* and subsequently were tested with *Salmonella pullorum* can be interpreted in at least two ways. (1) Resistance may be looked upon as due to a unit rather than a composite constitution, in which case the increased resistance to *Salmonella pullorum*, brought about by selection of resistance to *Salmonella gallinarum*, would be due to non-specific factors. Since, however, the resistance is not as complete with *Salmonella pullorum* as it was with *Salmonella gallinarum* it follows that the genetic basis of constitution must still be quite largely due to multiple separate factors or be composite. Or (2) since *Salmonella gallinarum* is closely related to *Salmonella pullorum* in a taxonomic sense it would be likely to have some like methods of producing disease as well as some unlike ones. The selection for resistance would consequently select for resistance to these like methods which would in turn give the selected stock a better chance of survival than the control stock lacking the genetic constitution for this resistance. The case could consequently be interpreted as indicating that constitution is a composite of numerous independent characters.

The data for the *Salmonella pullorum* resistant race cannot be interpreted as due to non-specific factors since the selected and control strains have no significant differences in their death rates. Differences in the technique of exposing the strains to disease might have a direct bearing on the problem in hand, however. Roberts selected for disease resistance by feeding his birds the standard dose by mouth. Lambert tested the resistant strain developed

by this mouth feeding by intraperitoneal inoculation, a different route. This factor, the difference in the body cells which would be called upon to resist the initial attacks of the organisms, could conceivably account for the fact that the birds selected for this one type of resistance showed but little resistance when another type of unselected cells were exposed to attack. Lambert has tested this question, however, and finds that the route of infection makes little difference in the survival rates when the doses are within the range used in the experiments.

Webster (6, 7) has studied the question of constitutional resistance from several different viewpoints. In one experiment mice which had survived a single but varied dose of five different organisms: mouse typhoid I; mouse typhoid II, *B. aertrycke*, *B. pestis caviae* and *B. paratyphosus B*; were injected by stomach tube with a standard dose of epidemic mouse typhoid strain II. The results of this experiment showed the survival value of the previously unexposed mice to be the least, 20 per cent; those mice previously exposed to bacilli of low pathogenicity, *B. pestis caviae* and *B. paratyphosus B.* next, 48 per cent surviving. The groups of mice exposed to bacilli of moderate pathogenicity, mouse typhoid II and *B. aertrycke* showed a still higher survival rate, 69 per cent and 73 per cent; the mice surviving bacilli of highest pathogenicity, mouse typhoid I, a survival rate of about the same value as that for mice surviving the attacks of moderately pathogenic organisms, 67 per cent. The order of this resistance for the different groups to the mouse typhoid II disease follows that of the pathogenicity of the previous organisms to which the mice were exposed rather than the agglutination relationship of these different bacterial species. From this fact Webster draws the conclusion that the resistance mechanism of the host contains important non-specific factors. From the viewpoint of immunology, as indicated earlier in this paper, this conclusion appears to be correct. It should not be carried over to the genetic concept of the host resistance to disease, however. The variations in the survival rates to the second inoculation seem to support, rather than otherwise, the view that the resistance in even such closely related diseases as those here studied is dependent upon quite specific genetic factors.

## RESISTANCE IN MAN

Other observations on variations in racial susceptibility to two or more injurious environmental agents seem to be confined to statistical studies of death rates of peoples of various racial groups. Dublin and Baker (8) have made one of the most extensive of these in their study of mortality of race stocks in the states of Pennsylvania and New York. Their data are presented in the form of age specific death rates from five groups of diseases, tuberculosis, cancer, organic diseases, pneumonia and Bright's disease. The death rates in these states of peoples derived by immigration from six different racial stocks, Irish, German, British, Austro-Hungarian, Italian and Russian are presented. The general sense of their findings, although not the specific differences, may be indicated by arranging the racial groups in order of their death rates, from those having the highest to those having the lowest, for each disease (table 2).

While it may be argued that the division into the different racial groups, Irish, German, etc., can be regarded as not more than an approximation to the genetic homogeneity of animals bred purposely for uniformity, the data distinctly argue for racial differences in susceptibility to these diseases. These racial differences

extend to more than one disease, in fact in the racial groups Irish, German and British they extend to all five diseases. It is this type of data which some have interpreted as indicating that genetic constitution for disease resistance may be rather simple, possibly a unit, in its factorial composition. Such a conclusion appears somewhat premature. A good deal of necessary evidence is yet to be gathered to establish such a view, i.e. it would at least require crossing of the racial groups and a study of the hybrids. The data as they stand are furthermore equally capable of having the disease resistance of the different races interpreted as due to the fortuitous combination of several genetic factors, one contributing resistance or susceptibility to one disease and another to another.

TABLE 2

| TUBERCULOSIS | CANCER | ORGANIC DISEASES | PNEUMONIA | BRIGHT'S DISEASE |
|---|---|---|---|---|
| Irish | Irish | Irish | Irish | Irish |
| German | German | German | German | German |
| British | British | British | British | British |
| Austro-Hungarian | Russian | Russian | Italian | Austro-Hungarian |
| Italian | Austro-Hungarian | Austro-Hungarian | Austro-Hungarian | Russian |
| Russian | Italian | Italian | Russian | Italian |

RESISTANCE IN PLANTS

The studies on plant diseases of bacterial and fungous origin also lead to the view that genetic constitution for disease resistance is of the multiple specific factor type. The degree of genetic complexity manifested by the given host disease relationship varies greatly depending both on the host and on the disease. Only briefest mention of the extensive data in this field can be made. One of the simplest host-disease relationship is that of fusarium wilt of cabbage studied by Walker (9). Resistance to the wilt was apparently due to a single major factor, resistance being dominant to susceptibility. This disease resistant factor was independent of other factors which made for resistance to other environmental agents. The case is much like the *piliformis* disease of mice which the writer has been studying. Here strain susceptibility is quite sharp and the major factor for resistance behaves as a dominant independent of several other resistance factors.

Resistance to anthracnose in the bean plant has been shown by McRostie (10) to be equally dependent on the genetic composition of the bean host and the anthracnose organism, *Colletotrichum lindemuthianum*. The alpha strain of anthracnose infects only plants homozygous for the recessive alpha gene. The beta strain of anthracnose infects only plants homozygous for another recessive gene. It takes the combination of these two genes in homozygous condition to make the plant susceptible to both strains. The two genes are independent in their inheritance. This particular disease and host relationship has been further extended by Burkholder (11), who isolated still another strain, gamma, of the anthracnose producing *C. lindemuthianum*. Host resistance to this strain is likewise dependent on a single mendelian factor. The variety of bean, Well's Red Kidney, which was resistant to both the alpha and beta types of *C. lindemuthianum* is susceptible to the gamma type.

Although the common rule seems to make factors for resistance dominant to

those for susceptibility this is not always the case. Dietz (12) working on rust resistance in oats showed that susceptibility to the rust infection in a cross between the varieties white Russian and Burt was dominant, the resistance being dependent on two factors, one of which inhibited the expression of the factor for resistance carried by the white Russian parent.

As with the animal disease studies, the reactions of different varieties of plants within a species may be classified into three broad categories: the plant may be naturally immune to the disease, showing no reaction on exposure to it; it may be resistant; or it may be susceptible, offering little opposition to the progress of the infection. Aamodt (13), studying the genetics of a strain of wheat, Kanred, immune to rust form 1, a strain which showed lesions of the disease but resisted its further encroachment, Kota, and a susceptible strain, Marquis, came to the conclusion that this difference in the reaction was due to three allelomorphic factors. Immunity was dominant to the resistant and susceptible allelomorphs.

Immunity or resistance to several physiologic forms of rust may be conferred by a single gene or it may limit itself to one form. This overlapping of the gene effects on physiologic forms of rusts may be such as to preclude combination of the resistance of one variety to different physiologic forms of rust with that of another variety to the physiologic forms to which it is resistant, one allelomorph of the pair making for resistance to one group of diseases and the other allelomorph making for resistance to another group of diseases. Such a case has been noted by Goulden, Neatby and Welsh (14). Their study of the reactions of the wheat varieties H-44-24 and Marquis lead them to conclude that variety H-44-24 was relatively resistant to rust forms 9, 14, 17, 21, and 34 but somewhat susceptible to 15. Marquis on the other hand was resistant to rust 14 and susceptible to 9, 15, 17, 21, and 34. These differences are held to be dependent on a single factor pair, the H-44-24 being homozygous for the RR resistant factor and the Marquis for the rr susceptibility allelomorphs. If this is true it then becomes impossible in this cross to combine the moderate resistance of the H-44-24 to rust form 21 with the high resistance of Marquis to rust form 14.

In oats the variety Victory has been shown by Gordon and Bailey (15) to be susceptible to six forms of the stem rusts. White Russian is resistant to forms 1, 2 and 5 but susceptible to 3, 4 and 6. Richland is resistant to forms 1, 2, 3 and 5 but susceptible to 4 and 6, while a selected strain of Joanette is resistant to 1, 3, 4, 5 but susceptible to 6. Definite differences in the genetic constitutions of the host are responsible for these differences. Attaining resistance to one form of rust by selection may evidently lead to susceptibility, not resistance, to another.

The same conclusion is to be derived from an examination of diseases less closely related than the physiologic forms of the rusts (Hayes, 16). Wheat has as serious diseases stem rusts, leaf rust, scab, bunt or stinking smut, loose smut and black chaff. Ceres, a selection from Kota Marquis, is resistant to stem rusts but is somewhat susceptible to leaf rust, bunt, loose smut and black chaff. Marquillo, a selection from Iumillo with Marquis, is resistant to stem rusts and somewhat resistant to leaf rust, black chaff and bunt, but susceptible to root rots. Certain selections from Eimer by Marquis crosses are resistant to stem rust, leaf rusts, bunt and loose smut but are susceptible to black chaff. These facts demonstrate the specific nature of genetic resistance in the host.

It is possible to turn the case around and say that the genetic constitution of the invading organism is the significant fact in

disease resistance. That the specific genetic composition of the disease producing organism is one of the variables in disease production has already been seen in the work of McRostie and Burkholder. It is equally strikingly demonstrated by the stem rust of wheat and oats as shown by Stakman (17) and his co-workers where numerous separable and stable physiologic forms have been distinguished. Attempts to alter these forms by outside agencies or to show them unstable have thus far failed. Their genetic constitution seems to maintain and to prescribe the limits of their pathogenic powers. If new forms arise they appear to arise as mutations of the same rare frequency as mutations in higher forms or to be the product of segregation of previously existing factors through hybridization and subsequent recombination as shown by the work of Newton and Johnson (18) and Caigie (19).

While the evidence here chosen for presentation is necessarily selected, as its volume precludes presenting it in full, it is believed to be a random sample of the available data. The data show the genetic constitution of the host to be a primary variable in the disease syndrome. The genetic organization of the pathogen has like importance. Evidently the genetic constitutions of both must properly fit together for the pathogen to invade the host and produce the characteristic clinical picture of the given disease. The comparison of the susceptibility of a population to two or more diseases shows that in the majority of critical cases the genetic constitution of the host is a composite of numerous independent factors some favoring resistance to one environmental agent and some to another, the fortuitous combination of these separate factors making the organism resistant to many or few such agents.

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# POLYEMBRYONY IN THE ARMADILLO: GENETIC OR PHYSIOLOGICAL?

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THE PROBLEM

THE nine-banded armadillo, *Dasypus (Tatusia) novemcinctus*, is of special interest to biologists, since it and the closely related *D. hybridus* are the only vertebrates which are known to exhibit specific polyembryony; i.e., in which the fertilized ovum regularly gives rise to more than a single embryo. Von Jhering pointed out, in 1885, that the females of hybridus gave birth to several young of the same sex, and surmised that they came from a single egg. In 1909 this supposition was given definite proof by Fernandez, and in the same year Newman and Patterson published a notice showing the occurrence of a similar phenomenon in novemcinctus. Patterson followed this in 1913 with a detailed account of the embryology (of novemcinctus) from the stage of the monodermic blastocyst through the origin and organization of the four embryonic primordia. In this paper Patterson noted his discovery of a delay in the implantation of the blastocyst, and stated that this quiescent period during which the vesicle lay free in the uterine cavity lasted at least three weeks. This statement was seized upon by other workers and was made the basis for a "physiological" theory of the cause of twinning, a theory whose superficial plausibility has largely camouflaged its vital deficiencies and contradictions. Briefly put, this theory is to the effect that the delay in development directly causes the twinning.

In a recent paper the writer has described the complete reproductive cycle of the Texas armadillo, *D. novemcinctus texanus*. The length of the quiescent period was found to be about fourteen weeks. This "free vesicle" stage was made the object of special study with the idea of evaluating the factors that were supposed to cause polyembryony. Some of the facts brought out by this investigation, the details of the budding of the embryonic primordia as described in Patterson's original paper, and certain work done on other mammals have all combined to cast strong doubt upon the validity of the physiological explanation for polyembryony, at least insofar as the armadillo is concerned, and to suggest that the time is opportune for a critical scrutiny of this theory and for an attempt to arrive at a rational explanation of twinning.

THE NEWMAN-STOCKARD THEORY

The occurrence in the same animal of a quiescent period in its embryology and of a constant form of polyembryonic development is a coincidence that could not fail to attract attention, and it is inevitable that the question should be raised as to the possible causal relationship between the two phenomena. The first published suggestion that polyembryony in the armadillo may be caused by interrupted development is found in Newman's *Biology of*

*Twins*. Newman suggested that the slowing down of development permitted secondary centers of growth to arise, these forming the primary and secondary buds which give rise to the embryo.

Stockard, in 1921, carried the theory forward another step by attributing the "loss of dominance" of the original axiation to a deficiency in the oxygen supply during the free vesicle period. Stockard's theory, reduced to its essentials, is as follows: The ovum of the armadillo lies free in the uterine cavity for some weeks during which time the lack of connection with the uterine mucosa brings about a deficiency of available oxygen. This lack of oxygen suppresses the original growing point on the blastoderm and allows new centers of proliferation to arise. When implantation finally occurs, the oxygen supplied allows development to be resumed; and the secondary growing points each form a primitive streak and, eventually, an embryo. Stockard was led to suggest a lack of oxygen as the causal factor from his experiments with Fundulus, in which a small number of double monsters were found when eggs were exposed to cold or crowded during their development.

The theory of discontinuous development as the causal factor of polyembryony was further elaborated by Newman in his *Physiology of Twinning*. Newman, although he criticizes vigorously certain points in Stockard's theory, adopts the latter almost without change. The principal difference between the two theories seems to be one of language rather than of thought. Newman not only restates the hypothesis that the delay in development is the cause of twinning, but he attempts to extend the theory to explain twinning in groups as alien to each other as the echinoderms, annulates, and vertebrates. We are concerned here with the theory only as it affects the armadillo, and I shall review only the part which bears on this animal.

The larger part of Newman's chapter on the armadillo is taken up by his discussion of "fission" versus "budding" as the means of separation of the embryonic primordia. This terminological skirmish seems rather pointless as it can have no possible bearing upon the cause of polyembryony. It should be said, furthermore, that the assumption on which Newman bases this whole argument is false. He objects to the term "budding" on the grounds that there is no common stock from which budding could take place; while, as a matter of fact, the common amniotic vesicle from which the embryonic primordia bud off is not only much larger than the buds, at the time they arise, but is actually larger than all four diverticula combined (see Patterson, 1913).

Newman places the immediate cause of twinning as a "partial loss of polarity or deaxiation" followed by "the physiological isolation of two, then four, growing regions" and the "independent development from the four growing points of four complete embryos," an idea which is essentially that expressed by Stockard. The conditions which bring about this supposed "physiological isolation" are a lack of "food and oxygen and a means of eliminating waste;" a lack which follows the failure of the blastocyst to attach itself to the uterine mucosa. Stockard's view is that the lack of oxygen is the principal factor. Newman, while admitting that this may play a direct part, believes that the true cause is the "failure to attain at the proper time the essential growth stimulus which is normally supplied by placentation." The failure of placentation is in turn ascribed to some abnormality of the corpus luteum, a suggestion that had already been made by Stockard. As Newman points out, we must next look for a

cause for the behavior of the corpus, and so on ad infinitum. The endless chain, however, is all the while getting further and further away from any relation to polyembryony, and it is profitless to attempt to pursue it in this connection.

Much of the difficulty in analyzing the value of this theory lies in the indefiniteness of the language used. Newman accuses Stockard of using "intangible morphological conceptions," citing as examples such terms as "discontinuous mode of development" and "developmental arrests;" yet, in the succeeding pages he makes use of the even more nebulous phrases "partial loss of polarity" and "physiological isolation of growing regions." The criticized "discontinuous development" used by Stockard is replaced by Newman with "cessation of development." The average reader will find little choice between the two theories, for merely redescribing a morphological process of development in new and unfamiliar language adds nothing at all to our knowledge of the causes of the process. To say that there is a "physiological isolation" of growing regions is merely saying in a roundabout way that more than one embryo develops from a single primordium, and does not answer the question as to why there are more than one.

Either despite or because of the intangibleness of the concepts expressed in the theory, this hypothesis of Newman's has gone practically unquestioned. This has been partly due to the lack of any wholly satisfactory explanation of twinning, partly no doubt to a mistaking of turbidity for profundity. So far as I have been able to find, the only denials of the validity of the theory have been made by Hamlett (1929), from a consideration of the badger, and by Fischer (1931), from a comparison with some European mammals. These criticisms will be considered in a later section.

## EVIDENCE FROM THE ARMADILLO

That the deficiencies of the uterine environment or the failure of implantation is the cause, directly or indirectly, of the stoppage of development of the ovum is implied or stated at various places in the papers of Stockard and Newman. This, however, is not true, as mitotic activity ceases some time before implantation would normally occur. Most of the blastocysts studied have come from the uterus, but Patterson and the writer have each obtained a few blastocysts which were washed from the tube. These vesicles average as large, and the cells of their inner cell masses are as numerous, as the average of uterine blastocysts. In other words, the development of the ovum comes to a standstill before it leaves the tube, at a time when the impending failure of implantation could affect the vesicles only if we credit the latter with a teleological power of foresight. Certainly this cessation of development cannot be due to the physiological effect of a missing stimulus whose presence is not yet due.

Not only is the quiescent stage initiated at the wrong time for the Newman-Stockard theory, but the actual conditions under which the vesicle passes this period are different from those postulated by the above mentioned workers. Both Stockard and Newman, the former in particular, have made much of the fact that the vesicle lies "free" in the uterine cavity, and have taken for granted a resultant lack of respiratory or excretory exchange as being responsible for the arrest of development and the loss of dominance or deaxiation of the supposedly single original axis. The deficiencies of this environment in respect to the possibility of exchange of gases

and metabolic by-products have been exaggerated due to a misconception of the actual conditions within the uterus. In the pregnant organ, the bloodvessels which supply its walls are engorged with blood and the whole uterus is turgid and saturated with plasma. The walls are thicker than in the non-pregnant animal, so that although the uterus is considerably larger in cross section, its lumen remains a small slit-like pocket filled with plasma in which the vesicle is bathed. The blastocyst is thus in as favorable an environment for respiratory exchanges or nutritive and excretory activities as is the average body cell, which has likewise only an indirect connection, through the intercellular plasma, with the blood stream. Furthermore, the blastocyst is not completely out of touch with the uterine mucosa. If a uterus containing a free vesicle be opened carefully the vesicle will be found actually in contact with the mucosa of the fundal area. There is, of course, no organic attachment; the blastocyst possessing merely enough adhesive power to retain its position. This adhesion is strong enough so that the vesicle sometimes does not come off in a salt solution until washed rather forcibly. The application of a fixing fluid, however, always suffices to separate the vesicle from the mucosa unless the trager cells have begun to function.

It is usually considered that a degree of relationship between trophoblast and mucosa as slight as described above is insufficient to allow the development of the embryo. It is true that in most placentates the ovum sooner or later develops a more intimate connection with the maternal bloodstream; but it must not be forgotten that in almost the entire group of the Marsupials we find conditions comparable to those described above for the armadillo. In the opossum the embryos are so loosely adherent to the uterus that if we make a short incision on either side the contraction of the uterine musculature is sufficient to expel every fetus in that horn. In spite of this seemingly inadequate provision for nutrition, the opossum embryo develops in a few days to a stage where it is able by its own exertions to travel from vulva to pouch, find a nipple, and attach itself. If the opossum ovum is able to proceed this far in its development, with no better system of obtaining food and oxygen than is found in the armadillo uterus early in pregnancy, then we cannot assume that it is the lack of these that causes the halt in its development. Some other factor must be sought.

In this connection, we should remember that even in the placentates there is at least one form, the pig, in which implantation never occurs. There is never any fusion between trophoblast and uterus, nor any erosion of the latter. Yet this lack of implantation does not retard development, nor does it induce polyembryony.

It may be pointed out here that out of the many hundreds of opossum eggs collected by Hartman, only one case of polyembryony was found—this in a form which should be extremely favorable for twinning if lack of implantation really plays any rôle as a causal factor, according to Stockard's interpretation of conditions in the armadillo.

Another point to be considered is the constancy of the process of polyembryony in the two species of Dasypus. In the nine-banded armadillo, the number of embryos is typically four. Variations from this number are very infrequent. In a series of 114 vesicles, old enough so that the number of embryos could be determined, one showed 5 embryos and one

showed 3. In a few vesicles in advanced development there may appear to be fewer than four embryos; these are usually cases of death in utero of some of the embryos. I have seen only one case of a vesicle showing two or three embryos where it was not possible to find the macerated or partially resorbed remains of the remainder of the set. The production of four embryos seems to be practically constant for this species. In a very few cases three or five embryos may be formed; there is no case known where less than three, or more than five, primordia were laid down.

The production of four (or rarely five) embryos is not the only constant feature about the embryology of *D. novemcinctus*; in this species the manner of origin of the primordia seems to be invariable. We may condense the following account from Patterson's description of the process. Soon after the hollowing out of the amniotic vesicle, we find this becoming elliptical and the floor of the cavity thickening at the two ends and thinning out in the center. These two thickened regions are the primary buds. Following this phase, a further shifting of cells of the primary buds gives rise to two secondary buds from each of the primary ones; these secondary buds, incidentally, have a definite position with respect to the original bilateral symmetry of the amniotic vesicle that was established with the appearance of the primary buds. The thickened ectoderm of the four secondary buds becomes the four embryonic shields of the four embryos.

In this species, the regular sequence of events in the process of budding off the embryonic primordia, the fixed orientation of these primordia to each other and to the original symmetry of the vesicle, and the almost invariable number of the embryos produced, are points which suggest that the mechanism of this development is under the control of some factor which is not easily swung aside from its predetermined course. Can this factor be a variation in the amount of oxygen available, or in the concentration of excretory products—factors which must vary widely according to the physiological condition of the maternal organism? Or can it even be the more uniform stoppage of development? A comparison with the conditions found in the other species of Dasypus will help us here.

In the South American mulita, *Dasypus hybridus*, Fernandez has shown that there are from seven to twelve embryos formed. As has been pointed out by Fernandez, and Newman calls attention to this in the earlier *Biology of Twins*, the embryos show absolutely no regularity in the manner in which they bud out from the ectodermic vesicle. Fernandez says on this point, "die Ectodermanlagen der Embryonen der Mulita ganz unregelmässig aus der primären Ectodermblase hervorwachsen, ohne jene bilaterale Symmetrie." Fernandez gives photographs of reconstructed models showing this point beautifully. His monograph is, unfortunately, not in wide circulation; but the interested reader can usually obtain the *Biology of Twins* which gives, on pages 74 and 75, outline drawings copied from Fernandez and showing the essential points mentioned above.

It is unfortunate that we know so little of the early part of the reproductive cycle in this species. Fernandez has never described a free vesicle stage in the mulita, and we have only his description of some ovaries of this animal on which to base our conclusions. In his first article on the embryology of the Edentates Fernandez calls attention to the fact that there is only one corpus luteum present. He then goes on to make the observation that the corpus must persist for a long time after

parturition, for it is found in animals killed a month and a half or two months before the beginning of pregnancy! In the light of what we now know about the early history of the ovum in the Texas armadillo, Fernandez' observation can only be interpreted as meaning that ovulation takes place some time before implantation, and that the mulita has a quiescent stage comparable to that in our species. If our inference is correct, we have two species with the same peculiar interruption of development, yet one of these species produces a quite variable number of embryos in an extremely haphazard relation to the common vesicle, while the other produces a much smaller, almost invariable number of embryos which always show a definite mode of origin from the ectodermic vesicle. Now no amount of "physiological isolation of growing regions" can explain why under the same conditions one species should have four embryos while the other has from seven to twelve. Neither can we understand why the manner of origin of these embryos should be so invariable in the one and so irregular in the other species if they are called directly into existence by the reaction of the blastocyst to a physiological condition which is apparently the same in both. Evidently these differences must be due to some intrinsic difference between the ova of the two species, or, in other words, to some hereditary difference between them; and this raises the suspicion that the whole process may be genetically controlled.

The fixity of polyembryony in these two armadillos is in marked contrast to the extreme uncertainty of its appearance under experimental conditions. I shall not attempt to analyze the work of Stockard on Fundulus or of Newman on Patiria, but shall merely point out that while these workers found it comparatively easy to cause abnormal development they were unable to induce the appearance of double monsters other than in an extremely haphazard and sporadic fashion.

In connection with the above mentioned regularity of the budding process as seen in the Texas armadillo, there is one feature which is in direct opposition to the hypothesis of arrested growth as the cause of multiple embryo formation. With the resumption of development following implantation, the ovum proceeds to the formation of a single amniotic vesicle whose structure gives no hint of its impending budding. This lag in the appearance of the embryonic anlage is not unprecedented, however, as this delayed effect has been described by Stockard in his experimental production of double monsters in Fundulus. The supposed effect of the quiescent period is seen in the differential thickening of the floor of the amniotic vesicle to form the two primary buds. Now according to the theory, this rearrangement of the structure of the vesicle to form two new growing points should be followed by the development of these points into two embryos, for the rearrangement of cells and the establishment of the new axes of symmetry is supposed to cause the ovum to return to a condition in which it is ready for development. Instead of this we find these two active embryonic regions each bifurcating without any antecedent pause in their growth. Even if the formation of the primary buds were induced by the pause in development during the free vesicle stage, this pause could not explain the origin of the secondary buds; for their appearance is preceded by the resumption of cellular activity and the complete rearrangement of the embryonic structures, and this must preclude any effect of the quiescent period being transmitted beyond the time of the first rearrangement

of the blastocyst's symmetry. If all four primordia appeared simultaneously as primary buds from the vesicle we should not be able to rule out their possible causation by the stoppage of growth; but a consideration of the actual time of their appearance eliminates the possibility of the Newman-Stockard theory applying to the origin of the definitive primordia.

### DELAYED IMPLANTATION AND POLYEMBRYONY IN OTHER MAMMALS

Despite the evidence presented in the foregoing pages, the strongest proof against the physiological theory of twinning comes from a comparison of the armadillo with certain other species. We already know of three placentates which resemble the armadillo in the presence of a quiescent period in their development, but which are not polyembryonic. In addition to these three forms, there is strong evidence for a similar history in eight species of martens and bears. This evidence may be found in papers of Ashbrook and Hanson and of Prell. We shall consider here only the species for which the embryology has been definitely worked out.

In 1854, Bischoff, in searching for the early stages of development of the European roe-deer, made the interesting discovery that the egg underwent an arrest of development during which it lay free in the uterine cavity. The arrest lasts from early fall until December, Bischoff describing it as occurring when the egg had reached the morula stage. As in all the deer, twinning occurs in the roe-deer, but is by no means the universal rule, and such twins are dizygotic. Bischoff's findings have since been confirmed by Keibel and others. This rather inconvenient exception to the theory of delayed implantation is explained by Stockard as not exhibiting polyembryony either because the stage when the delay takes place is not certain, although Keibel is quite definite on this point, or because of a "lack of tendency to form accessory embryo buds" (heredity). I have been unable to find a reference to Bischoff or Keibel in either of Newman's books.

The next reference to a delayed period in the development of the fertilized egg is found in a little known article by Fries, published in 1880. I have seen no reference to this work by either Stockard or Newman, and the results seem to be unfamiliar to most workers in this country. Fries was interested in the delayed fertilization described by several European investigators in the bats. He apparently reached the conclusion that the delay was in some way an adaptation to the hibernating habit, and in pursuing this line of thought he made a study of the European badger, *Meles taxus*, an animal which hibernates. The badger, however, proved to be unlike the bat and similar to the roe deer. Fischer has followed up Fries' discovery, and has recently published a complete account of the reproduction cycle. I give in the following paragraph a summary of Fischer's findings.

Meles breeds during the months of July and August; individuals killed at the end of that month or at the beginning of August have usually ovulated. Ova examined between then and December have not implanted, but lie free in the uterus, in a stage corresponding to the inner cell mass blastocyst of the armadillo. No embryos are to be found before December, implantation taking place during that month. The young are born about March 1. The number of corpora lutea and of ova was always larger than the usual number of young for the species, also the older vesicles observed have all been monoembryonic.

Fischer discusses the bearing of these findings upon Newman's theory as to the cause of twinning. He points out that both the badger and the roe deer exhibit a long delay in development, and yet polyembryony is not known to occur in either of these forms. Fischer concludes that Newman's hypothesis is untenable, and that twinning in the armadillo is the result of factors intrinsic in the egg.

The third species known to exhibit a free vesicle stage is the American badger, *Taxidea taxus*. The earliest part of the cycle is unfortunately still unknown, but it has been shown (Hamlett, '32) that unimplanted blastocysts are to be found throughout December and January, implantation taking place about the middle of February. A study of embryos from the primitive streak until just before birth shows the vesicles uniformly producing each a single embryo. Like Fischer, the author has pointed out (Hamlett, '29, '32) the significance of this reproductive cycle to the theory of twinning advanced by Stockard and Newman.

In the three cases cited above we have animals with delayed development which are not polyembryonic. Let us consider briefly the reverse of these cases. Besides the armadillos there is only one form known in which polyembryony occurs as a regularly occurring phenomenon; this is in the case of certain human stocks. It is known that in some families the production of single ovum twins follows the laws of heredity. It is unnecessary to review the evidence for this; those interested will find it presented and the literature reviewed in papers of Davenport and of Dahlberg. It has been shown that twinning, as well as the higher forms of polyembryony, is transmitted through either parent and that it will reappear in certain lines for generation after generation. In the light of all the evidence that has been accumulated, Newman's statement that the genetic theory of human twinning seems "fantastic" cannot be taken seriously.

Because of the situation presented by the armadillos, the question immediately arises as to the possible occurrence of delayed implantation or retarded development in cases of human twinning. Newman assumes that this retardation actually occurs. While our actual knowledge concerning the early embryology of human twins is, unfortunately, almost nil, we do possess certain statistical evidence that has a bearing on the topic under discussion. Not only is there no evidence that the length of gestation of twins is longer than the norm, but as a matter of fact, twins are very often born several days or weeks before the end of the usual two hundred eighty days. Some obstetricians place the percentage of these premature births as high as seventy-five or eighty per cent of all plural pregnancies. Under such conditions any period of delayed development at all comparable in length to those found in the armadillo or the other species named above would be incompatible with the production of viable children.

In view of the foregoing facts it is evident that we must recognize the association in the armadillo of discontinuous development and polyembryony as being purely fortuitous. The cases of the roe deer and the badgers show that polyembryony does not always accompany quiescence of the blastocyst, and a consideration of human twins renders it extremely unlikely that quiescence accompanies polyembryony in this species. Even in the armadillos there is seen to be a variation between species in the method and extent of polyembryony, while each species is relatively constant under varying conditions. In man we find that the occurrence

of one egg twinning is inherited, and that the factor may come from either parent. The exact number, and perhaps the mode of origin of the embryos varies from one species to another. Thus we find the Texas armadillo producing four young by a definitely oriented process of budding; the mulita producing a higher, variable number through irregular budding; and some lines of the human species producing twins, whether by budding or by some other process we are at present unable to say. The essential feature is that for each form it is a definitely determined process, not dependent upon hit or miss variations in the environment.

## DISCUSSION

The preceding pages have shown the inapplicability of the Newman-Stockard theory to the case of the armadillo, and the lack of agreement between the armadillo and the other mammals which show delayed implantation. It now becomes our problem to devise some explanation that will fit the facts already brought out and at the same time be as simple and logical as possible. In evolving this explanation there are three sets of comparisons that are of particular significance: the comparisons of the mulita with the nine-banded armadillo, of the two armadillos with twinning families in the human, and of the armadillos with such mammals as the roe deer and the badger.

In the first case, that of the mulita and the nine-banded armadillo, we find two closely related forms, each with a delay in its embryology and each reproducing by polyembryony; but the number of young and the relations of the embryonic diverticula differ in the two species, also the process is quite regular in novemcinctus and irregular in hybridus. That is to say, in these two forms the blastocysts develop under apparently identical conditions, yet they show differences which are as specific as any purely morphological features characteristic of the adults. Since these differences appear under identical physiological conditions and with the same interruption of development, it is evident that the different types of polyembryony found in these two species must be due, not to the physiological conditions under which the blastocysts develop, but rather to some inherent difference between the ova of the two species.

When we compare the species of Dasypus with human identical twins we see the case from another angle. Here we find both types developing polyembryonically, but one form has a long period of delay while the ova of the other show a continuous development. Here too we must invoke some inborn factor that will carry these eggs to a similar goal under widely different physiological conditions.

The reverse of this, finally, is seen when we compare our two armadillos with the roe deer or the badgers. Here we find a group of animals, all of which show an interruption in development, yet only the armadillos are polyembryonic. Once again we are forced to conclude that there must exist within the armadillo ovum some quality that causes it to give rise to quadruplets (or more, in hybridus), while those of other species under apparently identical conditions, show no slightest tendency toward twinning.

The foregoing three lines of evidence all unite in indicating that in Dasypus, and in certain human families, the ova possess some fundamental twist in their organization that causes them to develop polyembryonically irrespective of their physiological environment. Since this peculiarity of the ova reappears regularly in each generation, it fulfills the requisites of a hereditary character, and as such it is undoubtedly controlled by some gene or set

of genes. In the armadillos, where the two species under consideration seem to be homozygous for this character, verification of this by breeding experiments would seem to be impossible. In man, however, sufficient pedigrees have been collected, as noted previously, to confirm this conclusion. As is the case with the great majority of genes known, the reaction set up by this gene (or genes) is of sufficient intensity and stability so that it is not affected by environmental variations within the limits which allow development to take place at all.

The foregoing should not be interpreted to mean that all uniovular twinning is the result of definite hereditary forces. There are cases where an egg, designed by Nature to form a single embryo, has through some accident of cell division or of environment, through injury or death of cells, given rise to two or more embryos or parts of embryos. Such are the various types of double monsters and Siamese twins; doubtless some of these cases give rise to completely separated, normal embryos. Failure to recognize the accidental nature of these pathological cases has caused Newman to try to interpret the cause of the perfectly normal course of specific polyembryony in terms of the abnormal conditions which frequently accompany (and cause?) the production of double monsters.

Certain facts should be clearly understood at this point. Double monsters are unknown from the armadillo, although they should be very abundant if they are due to the same causes which produce the quadruplets. Double monsters are not found in human twinning families any more commonly than in the general population. Double monsters do not run in families as does twin production. The conclusion seems inevitable that the production of double monsters is not due to the same causes as is specific polyembryony. The first is accidental, specific polyembryony is the result of a definitely inherited genetic factor.

A word of explanation would seem due at this place in regard to the use of the term "physiological" in the title. The use of this word in distinction to "genetic" is not intended to imply that twinning is not associated with a profound modification of the usual physiology of development. Newman has unfortunately used the term (e.g., "Physiology of Twinning") as if it meant that the mechanism of development is so little fixed in the organism that it can be swung from monoembryony to polyembryony by mere changes in oxygen concentration or speed of development. It is the conclusion of the writer that polyembryony is dependent upon some genetic factor or factors, and is inherited as definitely as is, for instance, eye color. Just as eye color is dependent upon the physiology of the pigment-forming cells of the iris, so the series of cell divisions and cytotaxic shiftings that result in the formation of four embryonic primordia instead of one may be said to be an expression of the physiology of the developing blastocyst. But to pursue our reasoning a step further, just as the physiology of the iridial cells is directed by a definite gene (or set of genes) that determines that the cells shall or shall not lay down brown pigment, so the complex of physiological activities that we speak of as embryonic development is governed by definite genes that rule these activities as determinately and irreversibly as eye color in man or in fly is painted by its appropriate genes. We realize that eye color is due to the physiological behavior of the pigment cells producing certain morphological results; but we also know that this activity is determined by definitely inherited particles of chromatin; consequently, we think of eye color as

being the product of a genetic process. The same must be true of polyembryony. Newman's view is apparently that the genetic factors are negligible in determining the number of embryonic primordia produced; he says in one place that the idea of the inheritance of twinning is "fantastic." On the other hand, Newman is willing to believe that lack of oxygen, excess of carbon dioxide, the accumulation of wastes, or a variation in the rate of development is sufficient to so completely transform the course of development as to bring about polyembryonic growth. I feel convinced that the processes of embryo formation are so firmly ingrained in the organism that they are not capable of being affected by any variation in environment unless that variation is so profound as to completely upset the mechanism of development. The factors which Newman terms "physiological" may by their aberrations produce monstrosities; they can not bring about the regular and exact formation of several perfect embryos from the single egg as we see done in the armadillo. The physiology of twinning is not dependent upon external factors of the environment, it is governed by the intrinsic hereditary potentialities of the fertilized ovum.

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# NEW BIOLOGICAL BOOKS

***The aim of this department is to give the reader brief indications of the character, the content, and the value of new books in the various fields of biology. In addition there will frequently appear one longer critical review of a book of special significance. Authors and publishers of biological books should bear in mind that* THE QUARTERLY REVIEW OF BIOLOGY *can notice in this department only such books as come to the office of the editor. The absence of a book, therefore, from the following and subsequent lists only means that we have not received it. All material for notice in this department should be addressed to Dr. Raymond Pearl, Editor of* THE QUARTERLY REVIEW OF BIOLOGY, *1901 East Madison Street, Baltimore, Maryland, U. S. A.***

## BRIEF NOTICES

### EVOLUTION

THE FELIDAE OF RANCHO LA BREA. *Carnegie Institution of Washington Publication No. 422.*
*By John C. Merriam and Chester Stock. Carnegie Institution of Washington, D. C.* \$7.00 (paper); \$8.00 (cloth). 9 x 11¾; xvi + 231 + 42 plates; 1932.

In the Introduction to this fine addition to the Carnegie publications the authors say:

> Were one to select any single item to illustrate the most striking phase in the long list of exceptional features of our North American Pleistocene life as exhibited by the Rancho La Brea fauna, it would presumably be the representation of the Felidae. Excepting only the dire wolves, no group in the fauna of Rancho La Brea is represented by such a multitude of specimens, ranging up to more than one thousand individuals of the sabretooth cat and an exceptionally large number of individuals of the great lion-like cat, with a limited number of smaller types.

From this abundance of material, almost perfectly preserved in the asphalt deposits of Rancho La Brea, measurements of every skeletal part of representative individuals were made and are recorded in detail, both numerically in the text tabulations and pictorially in the excellent photographic and heliotype plates. Minute quantitative and qualitative comparisons are made both between specimens found in the Rancho La Brea and other North American deposits. For the comparisons, the various skeletal parts are considered collectively in separate sections of the book, but adequate cross reference numbers permit the study of all available measurements of a single individual animal. The book is thoroughly documented; it is beautifully printed; and the pen and ink and wash drawings by John L. Ridgway and the restoration drawings by Charles R. Knight are exceptionally well done.

DARWINS THEORIE DER GESCHLECHTLICHEN ZUCHTWAHL IM LICHTE DER HEUTIGEN FORSCHUNG. *Zugleich eine Untersuchung über das "Manometerprinzip" der Sexualselektion.*
*By N. G. Lebedinsky. Martinus Nijhoff, The Hague.* 13.60 guilder. 6⅝ x 9½; 244; 1932 (paper).

A review of the more important recent studies on the mechanisms operating in sexual selection. The following are among the more important conclusions.

> The secondary sexual characters attain their most luxuriant development, and the gonads and their products the greatest functional and healthy level, only with entirely normal metabolic constitution of the organism. This sensitivity of characters of an esthetic nature determines their inner selectional worth. Even though the females certainly choose their mates subconsciously, the fact that their taste is based on a "health and strength meter" facilitates a selection advantageous to race hygiene. Unconsciously the females choose healthy fathers for their offspring and thereby assure the success of species stability. [This is the essence of the "manometer principle."]
>
> The causes of the primary genotypic origin of esthetic sexual characters are, like those of mutation generally, unknown. Their appearance (whether it concerns entirely new characteristics or a functional change of already existing marks of differentiation of species and sex) in only one sex appears to be bound, at least in part, to differences in the general metabolism of the sexes.

This is an important book. It is equipped with a bibliography covering twenty pages, author and subject indices.

THE CEPHALASPIDS OF GREAT BRITAIN.
*By Erik A. Stensiö. British Museum (Natural History), London.* Three pounds. $9\frac{1}{2} \times 12\frac{1}{2}$; xiv + 220 + 66 plates; 1932.

This monograph based on material belonging to museums and private collections is of great interest and importance because of the antiquity and primitive characteristics of the cephalaspids, and their bearing on the evolution of vertebrates. A great many of the specimens are beautifully preserved with the result that it has been possible to work out the morphology with amazing detail and thoroughness. The work is liberally illustrated. The plates are superb. The first part of the memoir is devoted to a detailed discussion of the anatomy, and the second part to the taxonomy of the group. There is a final section comparing the cephalaspids with other ostracoderms. Altogether this is a contribution of the first rank to the literature of paleontology.

ASPECTS OF EVOLUTION.
*By F. W. H. Migeod. Heath Cranton, London.* 5s. net. $4\frac{7}{8} \times 7\frac{1}{2}$; 127; 1932.

The point of view and thesis of this book are contained in the following quotation from the final chapter:

> Evolution as a special theory has sunk from its position of being a mechanistic process of itself to being merely a paper record of accomplished facts. Creativeness is an all-pervasive force in nature, and creation is its outcome. In consequence, therefore, it would be difficult to recognize the creation of matter, life and mind without accepting a maker thereof, who may work by any of the various methods we find have been employed.

According to the author, mind is the chief factor through which change takes place. Habit, mimicry and homoplasy also assist. The author's sources appear to be almost entirely derivative, text-books and general treatises rather than original sources, but biologists may find a good deal of interest in reading a statement of the creationist argument, consistently and temperately developed. The author is an anthropologist.

DER WEG DES MENSCHEN VOM LINKS- ZUM RECHTSHÄNDER. *Ein Beitrag zur Vor- und Kulturgeschichte des Menschen.*
*By Richard Kobler. Moritz Perles, Vienna.* 7.50 marks (paper); 9.50 marks (cloth). $6\frac{1}{4} \times 9\frac{1}{2}$; ix + 142; 1932.

An attempt to prove that the prehistoric members of the human species were left-handed, and to trace the evolution of the transfer of [assumed] left-handedness to right-handedness. The author attacks the problem from several viewpoints, as indicated in the following chapter headings: The superiority of one hand—a characteristic of man; Prehistoric man—a left-handed person (based on a study of implements of the Stone Age); Right-handedness—a consequence of the use of weapons; The physiological foundations of right-handedness—critically viewed; Present-day left-handedness—a relic fauna; Left-handedness in the mirror of the cults; Cultural-philosopical views. The book is furnished with an eight-page bibliography, and author and subject indices.

## GENETICS

THEORETISCHE BIOLOGIE. *Erster Band: Allgemeine Theorie, Physikochemie, Aufbau und Entwicklung des Organismus.*
*By Ludwig v. Bertalanffy. Gebrüder Borntraeger, Berlin.* 18 marks (paper); 20 marks (cloth). $6\frac{3}{4} \times 10$; xii + 349; 1932.

A well-written and stimulating discussion of the present state of the fundamental concepts and problems of general biology. The author aims to summarize the existing theories in the various fields in the hope that this may aid toward the formation of an adequate general theory of life. The mechanistic, vitalistic, and *Gestalt* theories, taken separately, he shows to be inadequate. In this volume, after a critical

discussion of these theories, he proceeds to consider the physical chemistry, organization and embryonic development of living organisms. The author's philosophy leans, as is well known, towards the "organismal" position, and the temperate and scholarly development of the argument seems singularly sound and refreshing in a world bombarded with genes. This volume has its own bibliography.

## GENERAL BIOLOGY

GROWTH.

*By James L. Smith. Edited by J. S. Haldane. Oliver and Boyd, Edinburgh.* 6 shillings net. $5\frac{1}{2} \times 8\frac{3}{4}$; vii + 135; 1932.

Professor Smith, during the last years of his life, was engaged in the preparation of a book on growth, which, unfortunately, was left unfinished at the time of his death. The manuscript of the introduction and fourteen chapters, although unrevised, were in completed form, and together with a final summary chapter by the editor, are presented in this book. In discussing the author's purposes, Professor Haldane remarks, "Seeing clearly that the key to the problem of normal growth is the understanding of life itself, he had meant the book to embody the general conclusions which he had formed as to the nature of life, and the bearing of these conclusions on the abnormal forms of growth with which pathology deals." In another place the editor says, "I can confidently say that it was Lorrain Smith's ideal to make the distinctive conception of life the basis of pathology, just as it has been my own ideal to make this conception the basis of physiology." Taken at face value the book scarcely can be said to attain its ideal, or even to make a clear-cut consistent approach to it. It is, for example, far from clear whether growth is to be interpreted as meaning cell division, simple increase in cell size, or increase in the size of an organism by both enlargement and multiplication of cells. In the discussion of variation, height is considered to "approximate so closely to the average that stature is one of the most definite elements of human heredity." Certainly it may be questioned whether or not any scale of variation has been devised by means of which it may be shown definitely that one organic element is more or less variable than another. In the final chapter of the book, the theory is advanced, with scant reference to previous work, that malignant tumor cells arise as the result of injury to chromosomes which is transmitted from cell to cell as a mutation. There is a short list of references but no index. It is to be regretted that the opportunity was not taken to add to the book a bibliography of Professor Smith's publications.

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN. *Lieferung 401. Methoden der Süsswasserbiologie.* Containing following articles: *Das limnologische Laboratorium in Aneboda*, by Einar Naumann; *Die Untersuchung des Stoffwechsels der Wasserbakterien*, by Adolf Seiser; *Die Exkursionsuntersuchungen des Limnoplanktons*, by W. M. Rylov.

*Urban und Schwarzenberg, Berlin.* 8.50 marks. 7 x 10; 156; 1933 (paper).

Naumann's description of the limnological station at Aneboda, in southern Sweden, is meant to serve as a guide in planning small biological stations for the study of fresh water biology. There are numerous photographs and several ground plans of buildings.

Seiser discusses the cultural methods applicable to the water bacteria whose metabolism is so bizarre that they can live on hydrogen, carbon monoxide, paraffin, or phenol, as well as methods for the more prosaic forms that merely live on nitrogen, iron, sulphur or cellulose.

Rylov, of Leningrad, contributes a delightful article on what the well equipped biologist will carry when he goes on field trips to study fresh water biology. How many know that a folding chair, which can be carried in the indispensable *Rucksack*, is desirable? If you can get another person to carry it, you can take a collapsible boat with you, Rylov explains. We can heartily recommend this chapter to all unimaginative biologists who have never been on field trips but who think it might be nice to go sometime.

PHYSICAL CHEMISTRY OF LIVING TISSUES AND LIFE PROCESSES. *As Studied by Artificial Imitation of Their Single Phases.*

*By R. Beutner. The Williams and Wilkins Co., Baltimore.* $5.00. 6 x 9; x + 337; 1933.

Professor Beutner has revived an old dogma, that the way to understand a process is to make a model of it. There is a bit of the revivalist in his manner, including a firm conviction in his message, and an enthusiasm that is forever breaking out into whole paragraphs of italics. The use of analogy used to be called the preacher's fallacy; it is Beutner's hope that the use of physical analogies may become a major part of physiological research. He has made a collection of imitations of vital phenomena with the materials and equipment of chemical and physical laboratories. It falls under three main headings: models based on membranes, osmosis, and related forces; life processes related to crystallization or due to surface forces (here are placed respiration models and imitations of amoeboid movement); and a third section devoted to bioelectric phenomena. It is instructive to read through the indexes, the author index in particular, to see who is left out. Herrera gets a bare mention, MacDougal is left out, and the successful models of the ascent of sap are not in, either, although Gurwitsch and his mitogenetic rays receive a whole chapter, curiously enough.

THE ACTION OF THE LIVING CELL. *Experimental Researches in Biology.*

*By Fenton B. Turck. The Macmillan Co., New York.* $3.50. $5\frac{3}{4}$ x $8\frac{1}{4}$; xi + 308; 1933.

We devoted a good deal of space to the discussion of the late Doctor Turck's opinions in an earlier issue (Volume I, pp. 603–608) and this book does not show any essential change in point of view in the intervening time. It does, however, show two things: (1) If you believe in a theory strongly enough you can bring anything in human experience into accord with it; (2) Doctor Turck seems never really to have apprehended what a control experiment meant.

THE MECHANISTIC AUTONOMY OF NATURE.

*By Carl F. Krafft. Carl F. Krafft, 2510 Q St., N.W., Washington.* $1.00. $5\frac{3}{8}$ x 8; 101; 1933.

This book presents a discussion of the essential nature of living matter and life processes in which the Deity as a basic factor is definitely eliminated in favor of the "unalterable principles of geometry and statistical mechanics." On the whole it would appear that, as between Mr. Krafft's "spirazines" and Jahweh in the exegesis of knotty biological problems, honors are about even.

A FIRST BOOK OF BIOLOGY.

*By Mary E. Phillips and Lucy E. Cox. University of London Press, London.* 2s. 6d. net. $4\frac{3}{4}$ x $7\frac{1}{4}$; 270; 1933.

A simple introduction to biology for the young. The authors believe that the best approach is through nature study. Consequently no plant or animal is described that cannot be easily seen. The material is treated from a seasonal standpoint. As a further stimulation for children there are scattered through the book simple and easily executed experiments.

DONNÉES NUMÉRIQUES DE BIOLOGIE. BIOLOGY, *extracted from Vol. VIII (1927-1928) and IX (1929) of the* ANNUAL TABLES OF CONSTANTS.

*By E. -F. Terroine and M. -M. Janot. Gauthier-Villars et Cie., Paris.* 90 francs. $8\frac{3}{4}$ x $10\frac{1}{2}$; xxi + 129; 1932.

Volumes V, VI and VII have already been reviewed in Volumes II, IV and VI respectively of this REVIEW. Volumes VIII and IX deal with those biological constants reported for the years 1927 to 1929. A wide variety of phenomena are treated, as in previous volumes. The compiling and editing have been carefully done. The series is a useful one.

MERS ET OCÉANS.

*By Camille Vallaux. Les Éditions Rieder, Paris.* 20 francs (paper); 25 francs (cloth). $6\frac{1}{4}$ x 8; 100 + 60 plates; 1932.

An excellent brief account of the seas, their dominion and depth, movement and content, their effects on climate and on the lives of people involved in marine industries. The book is profusely illustrated with photographs of real aesthetic value. There is a bibliography of 25 titles.

## HUMAN BIOLOGY

LES FRAUDES EN ARCHÉOLOGIE PRÉHISTORIQUE *avec Quelques Exemples de Comparaison en Archéologie Générale et Sciences Naturelles.*

*By A. Vayson de Pradenne. Émile Nourry, Paris.* 75 francs. $6\frac{1}{4}$ x 10; 676 + 46 plates; 1932 (paper).

The greater part of this book consists of detailed and well documented narratives of frauds in prehistoric and general archeology. For comparison in the field of the natural sciences the celebrated case of Kammerer and *Alytes obstetricans* is presented. Having gathered his facts the author analyses them. The swindler and his motives, the dupe and the means by which he is ensnared, the unconscious leads which he gives the swindler for the development of the fraud, the specious arguments by which he supports the authenticity of the discoveries when they are called into question, are all analyzed with great skill and shrewdness. The final section is devoted to the technique of detecting fraud in archeology.

The book will be of undoubted value, not only to the archeologist, but to all who have the task of sifting truth from error. Much of it is, moreover, amusing reading, as, for example, the account of the 27,000 forged letters of such personages as Pontius Pilate, Mary Magdalen, Sappho, and Cleopatra which were bought for 140,000 francs by the celebrated French mathematician, Chasles. But perhaps the most amusing tale is that of the discovery in the valley of the Clyde of inscribed shells and other supposed prehistoric remains. The controversy over their authenticity raged for several years—Andrew Lang being one of their most impassioned defenders—until Boyd Dawkins discovered that the supposed prehistoric shells were those of Blue Point oysters and pointed out that either the importation of American oysters to Scotland began before the discovery of America or the finds were forgeries.

FLESH OF THE WILD OX. *A Riffian Chronicle of High Valleys and Long Rifles.*

*By Carleton S. Coon. William Morrow and Co., New York.* $2.75. $5\frac{3}{8}$ x 8; xi + 339; 1932.

This book tells a remarkable story, not alone entertaining to the reader, which would be a sufficient excuse for its writing, but full of interesting material for the thoughtful human biologist. For a young Ph.D. fresh from the university to trot serenely off to the upper bowels of the Moroccan Rif accompanied by a newly acquired and beautiful wife, live there all told more than a year, and come back still accompanied by the same wife, is to strain the credulity of those who do not believe in miracles. For the Riffians are tough babies, full of prejudices about strangers, particularly white ones, and nervous—not to say flighty—with the trigger finger.

However these amazing Coons got away with it. The technique of this book is to embody a great deal of sound Riffian ethnology, folklore and folkways into a connected account of the history of an imaginary family of the Vale of Iherrushen—a sort of Berber Forsyte Saga. And what a tale it is! The Riffian is a direct actionist. If he wants anything he goes and gets it; if someone annoys him it is just too bad for the widow and children. Inhibitions play but a small part in Berber behavior. And yet the account of the killing of a sister by her two brothers, because she had flaunted the code and slipped into ultimate amatory dalliance, shows how dreadfully rigorous the maintenance of standards of conduct may be among a people both unmoral and in many ways actively immoral.

We strongly recommend the book to our readers. It is cleverly and artistically illustrated with black and white drawings.

La Critica Medica nella Storia, Alessandro Magno.

*By Mario Bertolotti. Fratelli Bocca, Torino.* Lire 56. 6¾ x 9¾; 413; no date (paper).

The author has studied the biographies of Alexander the Great; the records of his physicians; and those of Eumenes of Cardia, his secretary and general, who, it appears, kept a more complete record of the Emperor's illnesses and wounds than his physicians did. This book is a notable and interesting reconstruction of his biologic, psychologic and medical history. The author deduces from descriptions and statuary that Alexander was a hyperthyroid type, and may have been afflicted with wryneck. He can find no evidence for the belief that he was homosexual. During his campaigns Alexander received nine wounds, the most serious of which—a fracture at the base of the skull and a chest wound involving the pleura but not the lungs—are described in detail. From records concerning the latter, the author deduces that he also suffered from empyema. In connection with the former, the author raises the question of Alexander's intemperance. He advances the theory that he gained this reputation in the period following the skull fracture by falling asleep at the many banquets he attended. This habit is assumed to have been due to hypersomnia as a sequel to the injury rather than to excess consumption of alcohol. Just conceivably, as a further possibility, banquets may have been as dull then as now. Alexander's irritability and suspicion of friends in his later years are ascribed to the influence of his intimacy with a group of soothsayers rather than to physical ills. Alexander had malaria twice. The first attack is compared with that of Byron. The description, as reconstructed from records, of the attack of the tertian type which caused his death, is splendid. The book is illustrated with many excellent photographs of statues, busts, and masks. A bibliography and a glossary of clinical terms are appended.

The Personality of Britain: *Its Influence on Inhabitant and Invader in Prehistoric and Early Historic Times.*

*By Cyril Fox. National Museum of Wales, Cardiff.* 2s. 6d. 8¼ x 10¾; 84 + 3 folding maps; 1932 (paper).

The prehistoric inhabitants of the British Isles and those who followed them left, quite unconsciously, remarkable gifts to the British anthropologist of today. So abundant and widespread have been the findings of the megalithic, bronze and early iron ages that it is now possible to predict with considerable accuracy where the early British types actually lived and labored and what determined their activities. In order to reconstruct the ancient picture comprehensively, however, it is necessary to take into consideration the profound differences in geography, climate, fauna and flora of megalithic times from the present day conditions.

In *The Personality of Britain* is given a general survey of all that investigation has revealed up to the present time concerning the different streams of culture which flowed into the eastern and western parts of the Isles and the effect which these cultures had upon those already established. In the highlands there tended to be a fusion of the invaders with those already established. In the lowlands the old culture tended to be replaced by that of the newcomers. The aloofness of Ulster from the general life of Ireland of today may have, according to the author, its basis in prehistoric times. Included in the work are many maps showing the distribution of the different types of early remains. The final map, in which there is an overprint of the distribution of antiquities of the Bronze Age, suggests that the British Isles may have been far more densely populated in that era than is generally assumed.

Indian Removal. *The Emigration of the Five Civilized Tribes of Indians.*

*By Grant Foreman. University of Oklahoma Press, Norman.* $4.00. 6 x 9; 415; 1932.

This is not a pleasant chapter in the development of our country. Five Indian tribes from the states of Tennessee, Georgia, Alabama, Mississippi and Florida, representing about 60,000 individuals, were forced to leave their lands to which they

were deeply attached and journey west, across the Mississippi, up the Arkansas River, into what is now Oklahoma. Their removal commenced in 1831. About ten years later the last group had been transferred. Intense suffering and hardship were endured by peoples who had not only long ceased to be nomadic, but had achieved a high degree of cultural development. Mr. Foreman has gone to the original documents, reports and letters, for his material. Not all of those who forced these tribes out of their homes or assisted in their emigration were blackguards. Particularly the officers and soldiers of the Army showed sympathy and consideration for their welfare. At every turn, however, there were those who were ready to take advantage of their helpless condition, either by bribery, corruption of their leaders with whiskey, or by downright fraud. The account is largely an exhibition of white ignorance, tyranny and cruelty. Illustrations and maps add to the interest of the book. It is carefully documented and indexed.

CRIMES AND CRIMINALS.

*By William A. White. Farrar and Rinehart, New York.* \$2.50 net. $5\frac{1}{2} \times 8\frac{1}{4}$; viii + 276; 1933.

The author has had wide experience in the practice of psychiatry and in dealing with mental patients from Federal prisons and military establishments. For nearly 30 years he has been in charge of St. Elizabeths Hospital, the government hospital for the insane, in Washington, D. C. A clear discussion is given of the fundamental principles "which must be taken into consideration in order that we may be able to think and act intelligently with regard to problems of criminology, be they the problems presented by crime in the abstract or by the criminal himself in the flesh." Dr. White holds that methods of punishment at present are largely a way of expressing what is at bottom vengeance and retaliation. Neither a prison, as it is now used, nor capital punishment are of any real value in crime prevention. All constructive work in the future must take into consideration the principle that destructive or dysgenic forces at work are offset by constructive or eugenic forces of equal strength. This principle, according to the author, has not generally been appreciated because the destructive force is so much more apt to be prominently in evidence. The first part of the book is valuable in clarifying the problem of dealing with anti-social people, and in showing what should not be done with our criminals. The latter part, dealing with penological principles and the trend of methods of crime prevention, is less satisfactory. Here we find much that is controversial. The book is briefly indexed.

HISTORY, PSYCHOLOGY, AND CULTURE.

*By Alexander Goldenweiser. Alfred A. Knopf, New York.* \$5.00 net. $6\frac{1}{4} \times 9\frac{3}{4}$; xxiv + 475; 1933.

This book represents many years of deliberation by the author on social theory. Most of the essays have appeared previously. All of these have been completely revised. The volume is arranged in six parts, as follows: Part One, *History, psychology, and culture.* A set of categories for an introduction to social science; The principle of limited possibilities in the development of culture; Psychology and culture; Anthropology and psychology; J. Teggart's approach to history; Culture and environment. Part Two, *Theories of primitive mind and culture:* Cultural anthropology, Sir James Frazer's theories; L. Lévy-Bruhl's theories; Wilhelm Wundt's theories; Sigmund Freud's theories. Part Three, *Totemism:* Totemism, an analytical study; The origin of totemism; Form and content in totemism; A final note on totemism. Part Four, *Religion:* Religion and society: A critique of Émile Durkheim's theory of the origin and nature of religion; Spirit, "Mana," and the religious thrill. Part Five, *Race:* Race and culture in the modern world; Are the races potentially equal? Part Six, *Variora:* Man and woman as creators; Is Freud a psychologist? Civilization as some school-children see it; The new education.

The book concludes with an extensive bibliography, an index of names and one of subjects.

THE PROBLEM OF LEMURIA. *The Sunken Continent of the Pacific.*

*By Lewis Spence. David McKay Co., Philadelphia.* $3.50. 5⅞ x 8½; 249; 1933.

Mr. Spence writes of that area in the Pacific which at one time was occupied by a continent, or several great land masses. It included New Zealand at one end and Hawaii at the other. Far to the eastward Easter Island with its remarkable monuments gives indications that it was in the past closely linked with the now sunken region, and may have been the Mecca of an ancient race. The geological evidence that such lands once existed seems good. The book deals chiefly, however, with the evidence presented by a study of the archaic temples and statues which are still extant, and remnants of culture and legends preserved by native islanders. The present Polynesian race has many legends which indicate that these ancient people were of white stock. The colored folk were probably employed in great numbers in their vast building operations, since huge stones were often transported many miles in temple construction. The existing hieroglyphic writings have never been interpreted. Included in the book are some entertaining theories concerning Lemuria which various writers of the air-castle type have advanced. The volume is interestingly illustrated, and indexed.

HISTORY OF AGRICULTURE IN THE SOUTHERN UNITED STATES TO 1860. *Carnegie Institution of Washington Publication No. 430. Volumes I and II.*

*By Lewis C. Gray and Esther K. Thompson. Carnegie Institution of Washington, D. C.* $6.25 (paper); $7.25 (cloth), for set. 6⅞ x 10⅛; xxviii + 1086; 1933.

This history of agriculture is a record of the continuous process of experimentation that went on in the pre-experiment-station era. These experiments involved not only finding crops adapted to the soils and climate of a new region, but also a perennial search for profitable markets; and on the part of legislators they meant adjusting subsidies and taxes, and dealing with the recurrent problem of over-production. It will be a surprise to many to find out how many things people had to do for themselves without governmental aid. It will also surprise other people to learn just how long and how successfully governmental agencies have had to come to the support of agriculture.

Any brief estimate of so important a book is bound to be inadequate. It is so smoothly written that one almost overlooks the thorough documentation. It is written as a monograph on agricultural economics but Dr. Gray has interpreted his field broadly and it ought to be helpful to anyone interested in any of the numerous crops or kinds of livestock that were introduced into this region. There is a helpful index.

SOCIAL BELIEFS AND ATTITUDES OF AMERICAN SCHOOL BOARD MEMBERS.

*By Claude E. Arnett. Emporia Gazette Press, Emporia, Kansas.* $1.75. 6 x 9⅛; xvi + 235; 1932 (paper).

The social beliefs and attitudes, with regard to economic, international, educational, and other like issues at the forefront in American life today, of 1,076 American school board members were determined by the questionnaire-test method. The findings are reported in this publication in the form of descriptive and comparative analyses by means of the statistical method. Results are stated in terms of "conservatism" and "non-conservatism." There is a short bibliography.

A BIBLIOGRAPHY OF THE HONOURABLE ROBERT BOYLE, *Fellow of the Royal Society.*

*By J. F. Fulton. Oxford (University Press). (Reprinted from the Oxford Bibliographical Society, Proceedings and Papers, Volume III, Part I, pp. 1-172).* 7⅛ x 10; 172; 1932 (paper).

This detailed and beautifully printed bibliography is a fruit of the author's interest in Boyle's medical and physiological works. However, it is not confined to these but deals with all his writings, scientific and theological. The preliminary notes to each book give a brief

analysis of it and the author hopes that the bibliography "in describing Boyle's works systematically, may pave the way for some one who is willing to undertake the formidable task of assessing his scientific achievements." The volume is illustrated by reproductions of Rysbrack's bust of Boyle and of the title pages of many of his books. There is an index. The vital statistician will be interested to note that Derham's *Physico-Theology*, the connecting link between the work of Graunt and Süssmilch, was originally delivered as one of the courses of lecture sermons "to prove the truth of the Christian religion against infidels" which Boyle had endowed in his will.

Handschrift und Ehe. *Eine Lehre vom Zusammenpassen der Charaktere, dargestellt an Handschriften aus Gegenwart und Geschichte.*

*By Bernhard Schultze-Naumburg. J. F. Lehmanns, Munich.* 4 marks (paper); 5.50 marks (cloth). 6 x 8¾; 117; 1932.

According to the publisher's blurb many of the 30 to 40 thousand unhappy marriages ending in divorce annually in Germany could have been easily avoided if the handwriting of the partners had been analyzed before the wedding. The author of this book has devised a method of presenting in schematic form the analyses of character traits based on Klage's system of graphology. He illustrates the book with samples and analytical diagrams of the handwriting of the husband and wife in a number of historically happy unions, and several recent unhappy ones, and compares the traits, as shown in the diagrams, for compatibility. By this method of comparison persons contemplating marriage can learn beforehand whether or not they will make good partners. We found the book more entertaining than convincing.

Musik und Rasse.

*By Richard Eichenauer. J. F. Lehmanns, Munich.* 7.50 marks (paper); 9 marks (cloth). 6 x 8¾; 286; 1932.

Hitherto the science of music has made use of the concept of race only when treating the so-called exotic arts of primitive peoples. In the work of Eichenauer we have an interesting and stimulating attempt to interpret the origin, character and development of the musical art of the West, on the basis of the various racial strains that enter into the make-up of the diverse European populations. The thesis is that it is important and necessary both for the musician and the music-lover to obtain a thorough knowledge of the science of race for a full understanding and proper appreciation of this art. The book is amply illustrated with portraits of representative masters and musical notations from their works. It is also provided with a subject index and an index of names of composers.

Rassenkunde und Rassengeschichte der Menschheit. *Erste Lieferung (Bogen 1-9). Zweite Lieferung (Bogen 10-18).*

*By Egon F. von Eickstedt. Ferdinand Enke, Stuttgart.* 10 marks each Lieferung. 7⅛ x 10½; Lief. I, 144; Lief. II, 145–288; 1933.

The first two numbers of a series of six purposing to summarize the present-day knowledge concerning the biology and history of the races and racial mixtures of man. After a preliminary discussion, contained in *Lieferung* I, of anthropological methods used in studies of race and type, and a general account of the origin and development of man and the races of man, the material is treated by continents. The second number treats of the peoples inhabiting Asia, from anthropological and biodynamic viewpoints. These numbers are replete with photographs, diagrams, and maps. Indices for the set will appear in the final number. When completed, it is plain that this will be a notable addition to the literature of general anthropology.

The Family. *A Study of Member Rôles.*

*By Katharine D. Lumpkin. University of North Carolina Press, Chapel Hill.* $2.50. 5½ x 8½; xix + 184; 1933.

This is a good book for the social worker

or those interested in sociological principles governing family life. Observations were made on forty-six families, clients of a family society in New York City. We are shown the endless variety of problems to be dealt with in family case work and offered excellent ideas on an approach to such matters as the various attitudes parents assume either to hide or to exaggerate a situation. We learn how the worker by understanding the main hidden motives behind an act can control a situation and build up a favorable attitude. There are also distinct family patterns, as for instance the wife who loyally assures the worker what a fine "provider" her husband has always been and in turn the husband who brags about what an excellent mother his wife is. However, with so much detail the reader has some difficulty in seeing the wood for the trees. There is an adequate index.

THE RACE BIOLOGY OF THE SWEDISH LAPPS. *Part I. General Survey. Prehistory. Demography. Future of the Lapps.*

*With the Collaboration of the Staff of the Swedish State Institute for Race Biology and of K. B. Wiklund. Edited by H. Lundborg and S. Wahlund. Almquist and Wiksell, Uppsala; Gustav Fischer, Jena; G. E. Stechert, New York.* Sw. Cr. 50 (Sweden); 50 marks (Germany); $15.00 (U. S. A.). $11\frac{1}{4}$ x 15; iv + 231; 1932 (paper).

This large monograph in folio represents the first volume of two on the Swedish Lapps to be published by the Swedish State Institute for Race Biology. Data for the study were derived largely from parish records covering approximately a century, from 1791 to 1890. Nearly one hundred pages of tabulated data give in detail all of the basic material used. The analysis is limited primarily to a comparison of the vital statistics and demographic characteristics of the two types of Lapps, the "nomadic" and "settled." The statistician for the Institute, Dr. S. Wahlund, furnishes an excellent and thoroughly sound evaluation of the errors inherent in the use of parish records. The general conclusions of the study are conservative and form a reliable and valuable contribution to the field of human biology.

TOLD AT THE EXPLORERS CLUB. *True Tales of Modern Exploration.*

*Edited by Frederick A. Blossom. Albert and Charles Boni, New York.* $3.50. $5\frac{7}{8}$ x 9; x + 425; 1931.

In spite of its considerable popularity, this symposium of contemporary travelers' tales cannot be regarded as a particularly notable or distinguished performance. Not only are the thirty odd chapters uneven in interest and treatment, as was to be expected, but some of the book is either rehashing or straight reprinting of already familiar stuff. Each chapter is prefaced by a thumb-nail biography of its author. The book is rather too sparsely illustrated with mainly excellent halftones. There is no index, an odd omission for this particular type of book. Its *raison d'être* is a worthy one—to make some money for the library of the Explorers Club.

FAKERS, OLD AND NEW. *A History of Cunning and Stupidity.*

*By Maurice Chideckel. The Stratford Co., Boston.* $2.00. $5\frac{3}{8}$ x $7\frac{7}{8}$; iii + 275; 1933.

Jazzy and superficial sketches of the gaudier religious imposters from Alexander of Aboniteichos to Alexander Dowie. For variety Mr. Chideckel mixes in a few murderers, literary forgers, pretenders, highwaymen and assorted rogues. The book is carelessly written. Thus the founder of the Catholic Apostolic Church appears as Irving Edwards. There is, of course, neither bibliography nor index. What might have been an interesting and valuable book becomes in Mr. Chideckel's hands merely a piece of cheap sensationalism. It seems a pity.

THE SKELETAL REMAINS OF EARLY MAN. *Smithsonian Miscellaneous Collections Volume 83.*

*By Aleš Hrdlička. Smithsonian Institu-*

*tion, Washington.* $2.25. $6\frac{3}{4} \times 9\frac{1}{2}$; x + 379 + 93 plates; 1930 (paper).

This accurate and very complete account of the known early skeletal remains of man will serve as a valuable reference work for all future anthropological investigation. Dr. Hrdlička has given in detail the history of each important find, its location and relation to other skeletal remains, and the geological formations of the region. Tables of measurements of all types are included and a chart showing the dimensions of teeth in early man. The volume is profusely illustrated with excellent photographs and drawings and is well documented and indexed.

Science and Human Life.

*By J. B. S. Haldane. Harper and Bros., New York.* $2.50. $5\frac{1}{2} \times 8$; viii + 287; 1933.

Dr. Haldane writes in his usual entertaining manner on a wide variety of subjects. As a sample we give the following: The scientific point of view; The inequality of man; Prehistory in the light of genetics; The place of science in western civilization; Physics declares its independence; My philosophy of life; What I think about; Birth control; Illnesses that make us healthier; and If Jesus lived today. The author's excuse for grouping these heterogeneous essays together is that a single idea permeated them, namely "How does science affect human life?" The volume lacks an index.

Compendio Statistico Italiano, 1932-XI, *Vol. VI.*

*Istituto-Centrale de Statistica del Regno d' Italia, Rome.* $5\frac{1}{2} \times 7\frac{1}{2}$; 334; 1932 (paper).

Besides the usual presentation of Italian statistics, this number of the *Compendio* contains the results of the census of 1931, a chapter on the economic progress of Italy during the first decennium of the Facist regime, and an appendix on still-births and infant mortality. In spite of the Facist emphasis on procreation as a patriotic duty the birth rate has fallen even more rapidly during the past ten years than before. Unfortunately the book lacks an index.

Anthropogeographie.

*By Otto Maull. Walter de Gruyter & Co., Berlin.* 1.62 marks. $4 \times 6\frac{1}{2}$; 136; 1932.

This little book of the well-known Göschen series gives an excellent concise account of the geography of man and his works. The author includes under anthropogeography demographic, racial, cultural, ethnographical and political factors. It is primarily intended for the general reader.

Bulletin der Schweizerischen Gesellschaft für Anthropologie und Ethnologie. (*Bulletin de la Société Suisse d'Anthropologie et d'Ethnologie*) 1932/33, *9. Jahrgang.*

*Société Suisse d'Anthropologie et d'Ethnologie, Zurich.* 3 francs. $6\frac{1}{2} \times 9$; 59; 1933 (paper).

## ZOÖLOGY

Colony-founding among Ants. *With an Account of Some Primitive Australian Species.*

*By William M. Wheeler. Harvard University Press, Cambridge.* $2.00. $5\frac{3}{4} \times 7\frac{3}{4}$; viii + 179; 1933.

On a trip to Western Australia in 1931 Professor Wheeler was able to work out successfully the method of colony formation in the Ponerine ants. These insects, commonly known as the bull-dog ants, exhibit a hitherto unexpected method of colony founding which links the behavior of ants with that of the solitary and social wasps. After the nuptial flight, the queens of this type establish their colonies independently in single cells in the earth. These cells are sealed but in some of the forms the queen apparently emerges at intervals for the purpose of foraging for food and cleaning out the nest. This important finding concerning the behavior of an ancient and primitive type of ant has led the author to review and discuss in detail the independent method of colony formation among all

the ants. He stresses the importance and bearing which studies of typical behavior patterns have in the biological sciences. The volume contains numerous illustrations, a lengthy bibliography and an index.

Three Kingdoms of Indo-China.

*By Harold J. Coolidge, Jr., and Theodore Roosevelt. Thomas Y. Crowell Co., New York.* $3.00. $5\frac{3}{4}$ x $8\frac{3}{4}$; 331; 1933.

This is a narrative account of Kelly-Roosevelts Expedition to the northern part of French Indo-China and Szechuan for the purpose of collecting vertebrate material, and especially large mammals, for the Field Museum. The Expedition, like the book, fell into two parts, the one headed by Harold Coolidge, and the other by Theodore Roosevelt. The zoölogical booty garnered was rich and varied, and the country traversed extraordinarily interesting. Considering the difficulties of climate and country there was relatively little illness. This was evidently due to the knowledge, skill, and painstaking care taken by the Expedition's physician, Dr. Ralph Wheeler. Unfortunately the good record was marred by the death of one member, Russell Hendee, who contracted a particularly malignant form of malaria while travelling apart from the others.

The book is richly illustrated with excellent photographs, and a few line-cuts that entertain by their crudity. It will delight all who love travel and adventure, and contains much of interest to the anthropologist and ethnologist, as well as the zoölogist. It is well indexed.

Étude Expérimentale de la Diapause des Insectes. *Supplément XV au Bulletin Biologique de France et de Belgique.*

*By Germaine Cousin. Laboratoire d'Évolution des Êtres organisés, Paris; Les Presses universitaires de France, Paris.* 75 francs. $6\frac{1}{2}$ x 10; 341; 1932 (paper).

A description of a series of experiments on the nature of diapause in the various stages in an insect's life, its influence on the growth and metabolism of the insect and the effects of several factors, such as temperature, humidity, and light on the diapause. *Lucilia sericata* Meig. was used in most of the studies, but some experiments were also performed on other diptera—*Phormia groenlandica* Zett. and *Calliphora erythrocephala* Meig.—and the hymenopteron *Mormoniella vitripennis* Walk. Under conditions that were constant and optimal for the physiological equilibrium of the organism the author found continuous development to occur, and concludes that diapause is not a necessary episode in the life of insects. The bibliography covers 17 pages.

Nature by Day.

*By Arthur R. Thompson. Ivor Nicholson and Watson, London.* 12s. 6d. net. $7\frac{1}{8}$ x $9\frac{1}{2}$; xi + 162; 1932.

There is as fine a collection of photographs of wild animals in this book as anyone could ask to see, not merely one picture of the representatives of a species, but often enough pictures so that one can get some notion of life history and habits. Most of his subjects (there are 122 photographs in all) are birds, but there is a very fair number of pictures of the smaller mammals, and of reptiles, fishes and insects. The text is a series of brief, informal sketches of the life histories of the diurnal British animals that an alert naturalist may hope to see, written for amateurs. There is no bibliography but there is a good index.

Up the Amazon and over the Andes.

*By Violet O. Cressy-Marcks. Hodder and Stoughton, London.* 12s. 6d. net. $6\frac{1}{4}$ x $9\frac{1}{4}$; 337; 1932.

"I had still lots to see. This was Peru; the land of the Incas, the land of Pizarro. Also I must see a doctor, and then joy! there was my mail of six months." This is one scrappy sample only of a rather scrappy account of the author's journey up the Amazon and over the Andes. The lady can travel and keep a personal diary, but the reader might like some continuity of thought and the scholar better grammatical construction. There are some

good points to the book, however; one being many excellent illustrations and another some useful advice to the novice in exploration: "Don't try to load a mule with tin boxes . . . Don't take china or glassware. Don't take cooking pots unless you wish to use them yourself, as the natives will not use them." The appendices also include lists of camp equipment and scientific instruments. A little information about vampire bats and the fishes of the Amazon is given in the preface. There is no index.

DIAGRAMS AND SKETCHES OF SOME INSECT VECTORS *and Other Arthropoda Injurious to Man and Beast. For the Use of Students. Second Edition.*

*By M. St. L. Simon. Garrett and Campbell, London.* 4 shillings. $9\frac{1}{2}$ x $11\frac{1}{2}$; 9 folding plates; 1932.

A series of excellent diagrams and drawings designed for students of human and veterinary medicine, as well as of entomology. There are altogether eight plates, with an additional chart showing the different genera of insects covered in each plate. The purpose of the volume is to save much of the time the student spends in drawing and making lecture notes. There is plenty of room on each plate for the student to annotate, change or emphasize any aspects of the drawings. A useful pamphlet, embodying a pedagogical idea new to us, and worthy of consideration in other fields of zoölogy and botany.

THE ANIMALS CAME TO DRINK.

*By Cherry Kearton. Robert M. McBride and Co., New York.* $2.50 net. $5\frac{1}{2}$ x $7\frac{3}{4}$; 219; 1933.

The author has spent 32 years in studying and photographing wild life in Africa. In this book he describes the "natural life of animals in Africa—particularly before that life is changed through the presence of the white man." An accurate observer, Mr. Kearton contributes much to our comprehension of what wild animals experience and endure in their natural habitat. It is unfortunate that his photographs have been so poorly reproduced. The book is without index.

TRAITÉ DE ZOOLOGIE. *Fascicule X et Dernier. Les Mammifères.*

*By Edmond Perrier and Rémy Perrier. Masson et Cie., Paris.* 45 francs. $6\frac{1}{2}$ x 10; 268; 1932 (paper).

Fascicule IV, on Birds, was reviewed in this journal in Volume VII, page 362. This volume follows the same plan as the previous ones, commencing with a general description of mammals, followed by a detailed discussion of their anatomy, physiology and morphology. The final section is devoted to a detailed classification of mammals. The present volume marks the completion of an excellent text and reference book.

A MANUAL OF FORAMINIFERA.

*By J. J. Galloway. The Principia Press, Bloomington, Ind.* $6.50 (cloth); $7.00 (buckram). $6\frac{1}{2}$ x $9\frac{3}{4}$; xii + 483; 1933.

A systematic description and classification of all known families, genera and species of Foraminifera, with an account of their distribution and habitats. This should be an exceedingly valuable reference work in micropaleontology.

AN ARCTIC SAFARI. *With Camera and Rifle in the Land of the Midnight Sun.*

*By Richard L. Sutton, Richard L. Sutton, Jr., and Emmy Lou Sutton. C. V. Mosby Co., St. Louis.* $2.25. 6 x 9; 199; 1932.

This narrative of a hunting cruise along the coast of Spitsbergen contains much information on the habits of arctic birds, seals, walrus and polar bears. There are many excellent illustrations, an appendix on Spitsbergen place names and a bibliography but no index.

THE OCCURRENCE OF STREPTOSTYLY IN THE AMBRYSTOMIDAE. *University of California Publications in Zoölogy, Vol. 37, No. 17.*

*By Theodore H. Eaton, Jr. University of California Press, Berkeley.* 25 cents. $6\frac{3}{4}$ x $10\frac{1}{2}$; 6; 1933 (paper).

POSTJUVENAL MOLT AND THE APPEARANCE OF SEXUAL CHARACTERS OF PLUMAGE IN PHAINOPEPLA NITENS. *University of California Publications in Zoölogy, Volume 38, No. 13.*

*By Alden H. Miller. University of California Press, Berkeley.* 25 cents. 6¾ x 10½; 22 + 2 plates; 1933 (paper).

## BOTANY

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN. *Lieferung* 399. *Ernährung und Stoffwechsel der Pflanzen.* Containing following articles: *Physikalische Methoden der pflanzlichen Lichtphysiologie*, by Erich Nuernbergk; *Die Analyse von pflanzlichen Wachstumsvorgängen*, by Erich Nuernbergk and H. G. du Buy.

*Urban und Schwarzenberg, Berlin.* 15.50 R. M. 7 x 10; 275; 1932 (paper).

In the first section of this handbook the discussion of light filters of various kinds should be of interest to workers in a number of fields, in particular the description of the Christiansen filter in which the wave length of the transmitted light depends upon the temperature. There are also some notes of general interest on temperature and humidity control, photo-electric cells, thermopiles and other instruments for the measurement of temperature and light. The apparatus used by Nuernbergk and du Buy at Utrecht for measurement of the responses of seedlings in monochromatic light of low intensities is fully described. There are several chapters devoted to recent methods for study of reflection, absorption, and transmission of light, particularly the ultra-violet, in plant tissue.

The contents of the second section deal with motion picture equipment used for automatic registration of the behavior of seedlings stimulated by monochromatic light. There are devices for making quantitative measurements of the plant response as recorded on the film. Quite aside from its interest to specialists in light relations there are numerous ingenious devices for measuring this and that which should appeal to the mechanically-minded.

A BRIEF SKETCH OF THE LIFE AND WORKS OF AUGUSTIN GATTINGER.

*By Henry N. Oakes. Cullom and Ghertner Co., Nashville, Tenn.* \$2.00. 6 x 9¼; 152; 1932.

Augustin Gattinger was born in Munich, Bavaria, on February 3, 1825. His education for the degree of medicine was undertaken at the University, from which he was fired, however, in 1849, because of attendance at student demonstrations working for a more liberal government. In fact, he expressed his principles so strongly that he was forced to leave immediately for America. Whether or not he ever acquired the legal authority to practice medicine the author never clearly states. Anyhow, Dr. Gattinger settled first in Charleston and later in East Tennessee; he had in each place a rather large medical practice. Not long after he came to America he commenced his botanical researches upon which his fame largely rests. He was an ardent collector, and spent a large part of his time in the woods where he discovered many new species of plants.

The biography as a whole is rather disjointed and unevenly written. The last 75 pages, devoted to excerpts from Dr. Gattinger's correspondence with leading botanists of the day, is the most interesting part of the book. The old doc worked at his plants with an enthusiasm and thoroughness characteristically German.

DIE SPALTUNGSGESETZE DER BLÄTTER. *Eine Untersuchung über Teilung und Synthese der Anlagen. Organisation und Formbildung sowie über die Theorie der korrelativen Systeme. Beitrag XVI zur synthetischen Morphologie.*

*By Martin Heidenhain. Gustav Fischer, Jena.* 30 marks (paper); 32 marks (cloth). 6¾ x 9¾; xii + 424; 1932.

Unfortunately, this book was written about thirty years too late to receive the attention that is due it. This does not mean that it is not a good book; it is. It merely means that fashions in scientific thought have so changed since Heidenhain began his work that one does not often encounter now the dogma that the

cell is the only biological unit of structure, and this is the notion that Heidenhain set out to controvert. For him, cell groups, and even tissue cavities, are partly independent, self-regulating units, and when they divide they usually do so dichotomously. For leaves, which he can treat as planes and which are thus much more suitable than the salivary glands on which he began, he has worked out a simple numerical means for describing the division of leaves into lobes, and it must be said that his system works very well on even finely divided leaves. Taxonomists might do well to have a look at this part. He set out to bring animal and plant anatomy into a common ground, and he succeeded but his book is for philosophically inclined biologists.

RECHERCHES SUR LA RESPIRATION DES VÉGÉTAUX.

*By Shri Ranjan. Imprimerie Régionale, Toulouse.* 12 shillings. 6 x 9½; 183 + 2 plates; 1932 (paper).

This is a thesis on the respiration of leaves. Ranjan is a former student of F. F. Blackman, and he has employed the experimental methods and the interpretations of his former teacher without making any essential changes in either. The section on anaerobic respiration is perhaps his best. Other sections deal with the influences of sugars, light and ozone. The whole book is characterized by the variety of methods and materials used rather than by thoroughness in any single part, but nevertheless his results deserve the attention of students of the subject.

FORTSCHRITTE DER BOTANIK. *Unter Zusammenarbeit mit mehreren Fachgenossen. Erster Band. Bericht über das Jahr 1931.*

*Edited by Fritz von Wettstein. Julius Springer, Berlin.* 18.80 marks. 6½ x 9½; vi + 263; 1932 (paper).

In treatment, this compilation stands midway between an abstracting journal and a textbook, and accordingly serves a very useful purpose. It is a collection of competently prepared, critical reviews of the botanical literature appearing during the year 1931, the several fields of botany being treated separately by specialists. It is proposed to issue further volumes annually, each covering one year's literature and they deserve to be widely read by botanists. There is no index, unfortunately; a bibliography follows each chapter.

MANUAL OF PLANT DISEASES. *Second Edition.*

*By Frederick D. Heald. McGraw-Hill Book Co., New York.* $7.50. 5¾ x 9; xii + 953; 1933.

The first edition of this standard text was noticed in Volume II, page 299 (1927) of this REVIEW. The important new researches since that time have been incorporated in this edition, especially those bearing on types chosen for detailed consideration. At the end of most chapters there is a list of important diseases, with references, which should be particularly valuable for the student.

WALDWEBEN. *Die Lebensgemeinschaft des deutschen Waldes in Bildern.*

*By K. Gerhard and G. Wolff. Hugo Bermühler, Berlin-Lichtwefelde.* 4.80 marks. 7¾ x 10; 128; no date.

In this beautiful book of photographs of the German forests, their trees and flowers, their mammals, birds, reptiles and insects, the authors aim to present the forest as a biological unit. The accompanying text is written with great charm. We can recommend the book to all nature lovers. There is an index.

TIETOJA METSÄNVILJELYS-TOIMINNASTA SUOMESSA, 1923–1930. *Silva Fennica 22.*

*By Erkki K. Cajandas. Society of Forestry in Suomi (Finnland), Helsinki.* 6½ x 9⅝; 35; 1932.

TUTKIMUKSIA KAASUTUHOISTA IMATRAN VALTIONPUISTOSSA. *Silva Fennica 23.*

*By Esko Kangas. Society of Forestry in Suomi (Finnland), Helsinki.* 6½ x 9⅝; 36 + 2 plates; 1932.

METSÄPATOLOGISEN TUTKIMUKSEN TEHTÄVISTÄ SUOMESSA. *Silva Fennica 24.*
*By P. S. Tikka. Society of Forestry in Suomi (Finnland), Helsinki.* 6½ x 9⅝; 24; 1932.

EHDOTUS MAATALOUSYLIOPISTON PERUSTAMISEKSI (*Proposals for the Establishment of an Agricultural University*). *Silva Fennica 25.*
*Society of Forestry in Suomi (Finnland), Helsinki.* 6½ x 9⅝; 94; 1932.

LAURI ILVESSALON MUISTO. *Silva Fennica 26.*
*By Lauri Ilvessalo. Society of Forestry in Suomi (Finnland), Helsinki.* 7 x 9½; 54; 1932.

ACTA FORESTALIA FENNICA 38. *Publications of the Society of Forestry in Suomi, Helsinki.*
6¾ x 9½; 416; 1932.

CONTRIBUTIONS DU LABORATOIRE DE BOTANIQUE DE L'UNIVERSITÉ DE MONTRÉAL. *No. 22: Études sur la Flore Algologique du Québec.—I.* by Jules Brunel. *No. 23: Contribution à l'Étude du Gentiana Victorinii*, by Jacques Rousseau.
*Institut Botanique, Université de Montréal.* 50 cents. 6 x 9; 26; 1932 (paper).

## MORPHOLOGY

MORPHOLOGISCHE UNTERSUCHUNGEN AN DER FIBULA DES MENSCHEN UNTER BERÜCKSICHTIGUNG ANDERER PRIMATEN.
*By Heinrich Sprecher. Orell Füssli, Zürich.* 6¾ x 9⅝; 162 + 14 plates; 1932 (paper).

Measurements were made in this investigation from nearly 500 fibulae, from 18 different human races, 9 orang-outangs, 8 gorillas, 8 chimpanzees and 10 hylobates. The technique used for taking the measurements followed that in the texts of Martin and Gieseler. No definitive correlations came out of the study and the author says in conclusion that the fibula shows a multiplicity of shapes; that there is little racial differentiation; and that the fibula can be of but slight use for purposes of race diagnosis.

DISSECTION OF THE CAT. *Practical Directions for the Dissection of the Cat and the Study of Its Anatomy, to Accompany Reighard and Jennings' Anatomy of the Cat.*
*By Jacob Reighard and H. S. Jennings. Revised, with the Addition of a Manual of Regional Dissection, by Rush Elliott. Henry Holt and Co., New York.* $1.25. 5½ x 8½; vi + 106 + folding chart; 1932.

A revision of the practical directions for cat dissection, first published by Reighard and Jennings in 1901, with the addition of a regional plan for dissection by Dr. Rush Elliott. It is hoped that the directions will prove more convenient for laboratory use in a separate booklet than they were before when incorporated in the *Anatomy.*

L'ŒUF HUMAIN ET SES ANNEXES.
*By Maurice Lucien and Henri Vermelin. Gaston Doin et Cie, Paris.* 35 francs. 6¼ x 9⅝; 157; 1933 (paper).

A useful concise manual, by the professors of anatomy and obstetrics at Nancy, for the student of obstetrics, summarizing the main features in the anatomy and physiology of human reproduction. It is clearly written, abundantly illustrated, and fairly well up-to-date, although some of the more recent American work is not adequately represented. It has neither index nor bibliography.

## PHYSIOLOGY AND PATHOLOGY

THE NEURAL ENERGY CONSTANT. *A Study of the Bases of Consciousness.*
*By John Bostock. George Allen and Unwin, London.* 6 shillings net. 5⅛ x 7¾; 178; 1931.

It is impossible to give an adequate conception of the author's theories concerning the development of human consciousness and the neural energy constant in a few words. Primitive consciousness (in the single cell) he holds to be pure awareness of heat, cold, etc. Formation of slowly discharging cells (a center for emotion) linked up with the 'awareness center' came about for the need of avoiding extremes of temperature and simple dangers. Comfort and discomfort became associated with the centers and later paramount needs such as hunger and reproduction. Subsequent

elaboration of somatic tissues and complicated modes of life led to the development of the organ of fine adjustment or discrimination. "Complete consciousness occurs only when the three centers of awareness, emotions and fine adjustment are in correct functional apposition." The extreme fatiguability of the neuron (the basal structure for all nervous activity) is taken care of by the enormous numerical reserve of neurons which serve their turn as needed. The neural energy involved is a constant. The author applies his theory to an explanation of sleep, dreaming and mental diseases. The work concludes with a bibliography of 61 titles and an index. Sir John Macpherson contributes a foreword.

MALARIA. *The Governing Factor.*
*By Elliot Fitzgibbon. C. W. Daniel Co., London.* 5 shillings net. $5\frac{1}{8} \times 7\frac{7}{8}$; 100; 1932.

Add one to the *Budget of Biological Paradoxes*. Like many another paradoxer the author writes with skill and subtlety, never letting the anti-vaccination and anti-vivisection cats out of the bag while he is writing about malaria. Indeed one very punditical journal reviewed the present volume in great seriousness, with all proper "on the one hand" and "on the other hand" stuff. The actuality of the matter is quickly set forth. The author, apparently a civil engineer who devotes a good deal of time and effort to maligning the art and science of medicine and its practitioners, is opposed to the idea that the mosquito is in any way involved in the epidemiology of malaria. His notion is that subsoil water is the real villain. We recommend the book heartily to teachers of epidemiology to put in the hands of their students as an exercise in detecting fallacious reasoning. It has no index, but nobody is likely to miss it.

LE RÔLE BIOLOGIQUE DE LA CATALASE DANS LE MÉTABOLISME D'ÉNERGIE.
*By J. H. Regenbogen. Gaston Doin et Cie, Paris.* 36 francs. $6\frac{1}{4} \times 9\frac{1}{2}$; 139; 1932 (paper).

Regenbogen has a high respect for the properties of catalase.

In the metabolism of energy catalase performs the task of protecting the anaerobic phase and of putting a check on the aerobic. The function of the ferment is then directed toward the liberation and destruction of $H_2O_2$, formed and bound by the peroxydase and not toward the rejection of the free $H_2O_2$ present in traces in the cells to the degree which produces metabolism, thus purifying the cells of a cellular toxin, and providing them the necessary molecular oxygen for cellular respiration.

Starting from this his speculations take him over a large part of the field of respiration and enable him to propound a theory of the rôle of insulin in diabetes. Given encouragement, he says, he is sure he can trace the influence of catalase in arthritis, gout, obesity, arteriosclerosis, skin diseases, and cancer.

A STANDARD CLASSIFIED NOMENCLATURE OF DISEASE.
*Compiled by The National Conference on Nomenclature of Disease. Edited by H. B. Logie. The Commonwealth Fund, New York.* $3.50. $4\frac{3}{4} \times 7\frac{1}{2}$; xvii + 702; 1933.

This nomenclature is the result of the collaboration between 27 national societies and governmental bureaus interested in medical, clinical and statistical projects, and the National Conference on Nomenclature of Disease. Previous nomenclatures have been considered in the development of this one which is more inclusive and takes into account both the etiology of the disease and the part of the body affected. The book is an effort to meet the need felt by many for a more accurate basis for mortality and morbidity statistics and for the recording of clinical results. The diseases are classified in a logical manner with the numbers of the International List of Causes of Death printed in italics for purposes of statistical comparison.

The book merits a good trial so that its faults, if any, may be corrected and an accepted standard nomenclature prevail.

CHEMICAL WAVE TRANSMISSION IN NERVE.
*By A. V. Hill. The Macmillan Co., New York.* $1.25. $5\frac{1}{4} \times 8\frac{1}{2}$; ix + 74; 1932.

This lecture was delivered before a group of chemists at Cambridge in the hope

that a Chemist or two may be induced thereby to come to the aid of Physiologists in one of the most difficult—and therefore the most attractive—of all scientific problems, the nature of the change . . . which is transmitted in nerve.

The nature and properties of the nervous impulse are set forth clearly and in detail from the point of view of the physiologist; and the possibilities of further researches indicated, in which the author earnestly bespeaks the assistance of the chemist, the physicist and the engineer. In a group of appendices additional data are given on: (1) the excitation of nerve, (2) the measurement of the heat production of nerve, and (3) the energy of a nerve stimulus. The author includes a list of references and an index.

La Transfusion du Sang de Cadavre à l'Homme.
*By Serge Judine. Masson et Cie, Paris.* 24 francs. 6⅝ x 9⅝; iv + 145; 1933 (paper).

A clearly written account of a new surgical therapy. The author, prompted by Professor Schamoff's experiments with dogs, developed successfully the technique of substituting blood from cadavers for that of living donors in transfusion. The substitution presents no new difficulties for doctor or patient and is in many ways superior. The purity of the blood is assured by autopsy. One corpse yields enough for five to eight cases. The blood is easily conserved for more than fourteen days and consequently is ready for use at any time. In addition Professor Judine believes the blood of cadavers to have a sterilizing effect on the patient's blood that aids in fighting infection, causes a fall in temperature, and raises the tonus of the body.

The Organs of Internal Secretion. *Their Diseases and Therapeutic Application. With a Chapter on Obesity and its Treatment. Fourth Edition.*
*By Ivo G. Cobb. William Wood and Co., Baltimore.* $3.50. 5½ x 8½; xiii +303; 1933.

With the exception of the chapter on the Endocrine Glands and Nervous Disorders this book has been completely revised to take into account the important additions which have been made on the functions of the internal secretory organs since the third edition was published. Two chapters, one on Obesity and one on Infantilism, have been added.

The History of Dermatology.
*By Wm. Allen Pusey. Charles C Thomas, Springfield, Ill.* $3.00 postpaid. 5⅝ x 8¼; xiii + 223; 1933.

This book represents a real contribution to the literature of medical history. It is a scholarly piece of work, covering developments in dermatology from the Egyptians to the present time. The author's outlook is broad and his literary style delightful. Besides a general index there is an historical index. There are excellent illustrations.

The Tides of Life. *The Endocrine Glands in Bodily Adjustment.*
*By R. G. Hoskins. W. W. Norton and Co., New York.* $3.50. 5½ x 8½; 352; 1933.

An authoritative, well written, up-to-date, popular account for the general reader of the high points of present day humoral physiology, illustrated, documented, and indexed.

## BIOCHEMISTRY

Tissue Proliferation and Acid Base Equilibrium.
*By Rudolf Bálint and Stefan Weiss. Translated by F. Morena with the assistance of G. C. Pether. Constable and Co., London.* 18 shillings net. 6 x 9¾; ix + 211; 1932.

In Chapter I of this book, the problem of acid base equilibrium and its relation to inflammation in general is discussed, and the literature in the field briefly surveyed. Arguments in favor of the view that an important rôle is played by the displacement in the acid direction of the reaction of the blood and the tissues in the pathogenesis of ulcer are presented in Chapter II.

The other chapters of the book are concerned with the general connection existing between physiological and pathological processes and the regulation of the physicochemical reactions. The results of research into tuberculous inflammation, of tissue culture experiments *in vitro*, and of experiments on physiological and pathological growth all indicate that an acid reaction hinders, while an alkaline favors, all kinds of tissue proliferation. The book contains subject and name indices and a full bibliography.

HYDROCHEMISCHE METHODEN IN DER LIMNOLOGIE *mit besonderer Berücksichtigung der Verfahren von L. W. Winkler. Die Binnengewässer, Band XII.*

*By Rezsö Maucha. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.* 18 marks (paper); 19.50 marks (bound). $6\frac{5}{8}$ x 10; x + 173; 1932.

The quantitative chemical analysis of the waters of streams and lakes for ecological purposes has necessitated the development of a number of special methods which could be applied at the place where the samples were taken with simple apparatus in a minimum of time, and Winkler has been a leader in this kind of work. One of his students, Doctor Maucha, has compiled a well-written manual of analytical procedures of this type, well-indexed, and provided with nearly 150 literature references. The methods for measuring oxygen and carbon dioxide take up about half of the book, another section is devoted to the estimation of electrolytes, and the last part concerns dissolved organic substances.

SÄURE-BASEN-INDICATOREN. *Ihre Anwendung bei der colorimetrischen Bestimmung der Wasserstoffionenkonzentration. Gleichzeitig vierte Auflage von "Der Gebrauch von Farbindicatoren."*

*By I. M. Kolthoff in coöperation with Harry Fischgold. Julius Springer, Berlin.* 18.60 marks (paper); 19.80 marks (bound). $5\frac{1}{2}$ x $8\frac{3}{4}$; xi + 416 + folding plate; 1932.

A standard work by an authority in the field, revised and published under another title. The first two chapters, on the measurement of acidity, and the reaction of the ampholytes are essentially the same as in earlier editions. The chapter on the use of indicators in neutralization analysis has been omitted in this edition, as the author has treated this fully in his *Massanalyse*. The remainder of the book has been entirely rewritten and greatly expanded. Author and subject indices are provided.

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN. *Lieferung 400. Allgemeine chemische Methoden. Die Methoden der Diensynthese.*

*By Kurt Alder. Urban und Schwarzenberg, Berlin.* 13.50 marks. 7 x 10; 212; 1933 (paper).

Organic compounds with two pairs of double-banded carbon atoms each may be made to combine by addition and this handbook contains many procedures of this kind. It is the last one of the series on general chemical methods and contains the index for the series.

EIN GANG DURCH BIOCHEMISCHE FORSCHUNGSARBEITEN.

*By Arthur Stoll. Julius Springer, Berlin.* 3 marks. $5\frac{5}{8}$ x $8\frac{1}{2}$; 41; 1933 (paper).

This review, which is based on a lecture read at a meeting of the *Vereinigung Schweizerischer Naturwissenschaftslehrer*, is limited primarily to discussions of recent contributions to the chemistry of chlorophyll, ergot, squill and some of their derivatives.

FILME UND FÄDEN. *Haupt-Vorträge. Gehalten auf der IX. Hauptversammlung der Kolloid-Gesellschaft in Mainz vom 28.–30. September 1932.*

*Wo. Ostwald [Editor]. Theodor Steinkopff, Dresden and Leipzig.* 12 marks. $7\frac{3}{4}$ x $10\frac{3}{4}$; 183; 1932 (paper).

A collection of the principal papers—with the exception of that by L. Michaelis, which will be published in a later number

of the *Kolloid-Zeitschrift*—presented at the 1932 meetings of the Kolloid-Gesellschaft. Those not originally presented in German have been translated.

## SEX

SEX AND BIRTH CONTROL.

*By Leon F. Whitney.* \$1.50. $4\frac{7}{8} \times 7\frac{3}{8}$; 71; 1932.

THE TECHNIQUE OF CONTRACEPTION. *An Outline.*

*By Eric M. Matsner.* 50 cents. $5\frac{7}{8} \times 8\frac{3}{4}$; 39; 1933 (paper).

What a people the Americans are! If a law is found to be inconvenient or unpleasant they promptly devise ways and means of getting around it. A combination of glad-hearted and naïve hypocrisy with great ingenuity of mind characterizes equally our attitudes towards such a simple and homely physiological matter as copulation, and such grave concerns as our foreign policy and the soundness of our currency.

There is a plethora of laws in this country forbidding or restricting the dissemination of birth control information to the lay public. But almost daily new books and pamphlets appear giving such information in ever greater detail and specificity. Mr. Whitney's book, written by a layman, and dedicated to "the Mothers and Fathers who will someday be the ancestors of a better America," has on the reverse of the page carrying the dedication, the following statement: "This book is for married people only. If you obtained a copy and you are unmarried, the agent who sold it to you violated the agreement with the publisher." Oh yeah! And then what?

Dr. Matsner's pamphlet is stated to be issued in response to a "demand from the medical profession," and as "a convenient summary for the busy physician," but, on the inside cover page the price is quoted at 20 cents a copy in "lots of ten copies," as against the single copy price of 50 cents. "Busy physician" my eye—in fact my singularly bilious eye!

The moral fervor of both of these treatises is high and noble; their physiology impeccable and fully illustrated. What more need we say. Under the law we are forbidden to state where or how either book may be obtained.

LOVE. *An Outspoken Guide to Happy Marriage.*

*By X-Ray. The C. W. Daniel Co., London.* 1s. net (paper); 2s. net (cloth). $4\frac{7}{8} \times 7\frac{1}{2}$; 55; 1932.

It was with relief that we read this simple, concrete exposition of the fundamental biological and psychological facts of sexual development. Unlike many of its kind, the book neither moralizes, nor enlarges on the "art" of love-making, nor sentimentalizes to a distressing degree. The viewpoint is that of sound mental hygiene. The book is not written for scientists, but should find a place in the hands of those with limited knowledge about the facts of sex.

## BIOMETRY

STATISTICS *in Theory and Practice.*

*By L. R. Connor. Isaac Pitman and Sons, New York.* \$3.75. $5\frac{3}{4} \times 8\frac{3}{4}$; xvi + 371; 1932.

This is a clearly and simply written introductory textbook. Part I, on statistical method, deals with the organization of a statistical inquiry, statistical data, statistical measurement, classification and tabulation, diagrams, graphs, derivative data, statistical groups, averages, dispersion, skewness, probability and error, sampling, correlation, index numbers, finite differences, interpolation, graduation and curve fitting. Part II deals with the sources of data for administrative, economic and business statistics and with practical applications of method. The value of the book is enhanced by a bibliography and an index.

LES PRÉLUDES ANTIQUES DE LA THÉORIE DES PROBABILITÉS.

*By K. -G. Hagstroem. C. E. Fritzes, Stockholm.* 6 kronor. $6\frac{1}{4} \times 9\frac{3}{4}$; 54 + 3 plates; 1932 (paper).

According to the historians of mathematics

the concept of mathematical probability dates back only to the fifteenth century. Hagstroem concludes, however, that the concept of degrees of probability which the Greek philosophers of the Sceptic school introduced foreshadowed the modern concept of subjective probability and that the Greek game of astragali shows, in the values assigned to different throws, a knowledge of their objective probabilities.

KÖRPERFORM UND LEISTUNG SECHZEHNJÄHRIGER LEHRLINGE UND MITTELSCHÜLER VON ZÜRICH. *Eine sozialanthropologische Untersuchung.*

*By Ernst Biedermann. Orell Füssli, Zürich.* $6\frac{3}{4}$ x $9\frac{5}{8}$; 84; 1932 (paper).

Two hundred boys attending trade schools and the same number attending commercial schools, all between $15\frac{1}{2}$ and $16\frac{1}{2}$ years of age, are compared with respect to athletic performance tests and somatological characteristics. In general, the results are in accord with many similar studies of the differences between children whose parents fall in different socio-economic and occupational classes. An appendix gives all of the actual data on which the paper is based together with the statistical constants, mean, standard deviation, range and coefficient of variation.

MASS UND ZAHL IN DER PATHOLOGIE.

*By Robert Roessle and Frédéric Roulet. Julius Springer, Berlin and Vienna.* 16 marks (paper); 17.40 marks (bound). $6\frac{3}{4}$ x $9\frac{5}{8}$; vii + 144; 1932.

The authors have summarized in this small volume a vast amount of numerical data on weights and measurements of normal organs and organ systems. The material is collected largely from the literature; tabulations are made specific for age and sex; and in general, the number of cases, the arithmetic mean, the standard deviation, and standard error of the mean are given. To those interested in quantitative pathology this is an invaluable handbook.

## PSYCHOLOGY AND BEHAVIOR

CASE STUDIES IN THE PSYCHOPATHOLOGY OF CRIME. *Volume One, Cases I–V.*

*By Ben Karpman. Mimeoform Press, Washington.* $12.00 net. $8\frac{1}{4}$ x $10\frac{1}{2}$; x + 1042; 1933.

The merit of this formidable treatise lies in its thoroughness. This becomes evident when it is perceived that 1039 solidly set large pages are devoted to the case-histories of *five* (only) individuals. Probably never before have psychiatric cases been reported (in print) with anything like such completeness of detail. The case material comes from the Department for Criminal Insane (Howard Hall) at St. Elizabeths Hospital. The purpose for which the colossal labor represented by the book was undertaken was "to gain an understanding of some problems of criminality through an intensive study of the lives of individual criminals, seeking particularly to uncover such psychogenetic factors as may be found behind the criminal reactions proper."

While the thoroughness of the work commands great respect the net results achieved seem hardly worth the trouble taken. The author attempts no general conclusions whatever. In essence the five cases come to about the following: Case 1 was a hypersensitive chap with an inferiority complex whose criminal behavior (never particularly important) arose from "overcompensation for a deep sense of defeat;" Case 2 belonged "to that class of drifters whose life is one long childhood;" Case 3 was a homosexual male prostitute who got caught in the practice of sodomy in the Army—*why* he was homosexual does not appear; Case 4 seems to have been just a jackass, without brains enough to keep out or get out of trouble however slight; finally Case 5 was able "to distinguish intellectually between right and wrong, but unable to choose the right thing emotionally."

In sum, Dr. Karpman appears to have succeeded in really understanding each of these five men, and the reasons for their individual difficulties. But, broadly speaking, what of it? Surely it is not a discovery to find that personalities defective neurologically or psychologically, or both, may inadvertently drift into crimi-

nality. Like Herbert Spencer and the horse race, 1039 pages of indubitably dull reading are not necessary to convince any sensible man of a conclusion so plain. In fairness it should be said, however, that the book will doubtless help to convince people that *punishment* offers no solution for such cases as these, either from the viewpoint of society or of the individual concerned.

The index of the book appears to us inadequate, even in spite of the author's defense of it.

MAGIC AND MIND.

*By E. J. D. Radclyffe. The Macmillan Co., New York; A. and C. Black, London.* $1.00 (U.S.A.); 2s.6d. net (England). $4\frac{7}{8}$ x $7\frac{1}{4}$; 96; 1932.

We are accustomed, in our superior wisdom, to smile at the belief of the savage that his magic spells can bring rain or heal sickness. Yet, as Mr. Radclyffe points out, however mistaken the technique may have been, the conviction that man can control his environment is of vast importance. It is indeed the basis on which science and technology have been built up.

But unfortunately for mankind the road from magic to science was not a direct one. To learn the effective control of the environment is a long and difficult process. It is little wonder, therefore, that after the first stage of man's overcredulous conviction of his own powers there should have come a second stage when

> He began to believe himself a weakling and to become aware of great powers at work other than his own. He might have trusted them and been content; only too often he feared them, to the ruin of his peace of mind. He learned first uncertainty; then terror, guilt, and the sense of sin. He ceased to reverence himself, and wasted his reverence on others. He sacrificed his flocks, and even his children, his self-respect, and his manhood, to misconceived gods, his strength of body to power-loving kings, and his wits and confidence to mistaken priests.

In our relations with the physical world this stage is, in turn, passing. The physical sciences have given us a large measure of control over our external environment. But in our inner life and our relations with our fellow men the baneful influences of fear and taboo still linger. The author has hope of the development of a "science of living" which will not quarrel with our make-up and deny its exhibition but will have the courage of our nature, accept it, and arrange society around it.

THE SECRET OF LAUGHTER.

*By Anthony M. Ludovici. The Viking Press, New York.* $1.75. $4\frac{3}{4}$ x $7\frac{1}{4}$; 134; 1933.

After a discussion of the various theories of laughter that have been proposed the author adopts as the basis of his own theory Hobbes's conclusion that "the passion of laughter is nothing else but *sudden glory* arising from some sudden *conception* of some *eminency* in ourselves, by *comparison* with the *infirmity* of others. . . . ." Laughter, for Mr. Ludovici, is the expression of superior adaptation; its origin is the baring of fangs by which animals warn off their foes. With the increasing use of external weapons the showing of teeth, while retaining its instinctive association, the expression of superior adaptation, was "transferred to all those manifold and complex situations in society in which gregarious animals either find or feel themselves superiorly adapted, or merely lay a false claim to such a position by means of bluff."

The craving for laughter of the present age is in Mr. Ludovici's eyes a symptom of its decadence. Mankind, oppressed by physical ills as well as by the complexity of the environment, resorts to the factitious and transitory superiority of laughter, instead of seeking to remedy its inferiority. Thus humor, like religion, plays the part of an opiate of the people.

This is a stimulating and provocative book. It is to be hoped that some of its inevitable opponents will accept the author's challenge to account for the facts by a theory as all-embracing as his. The book is well documented and contains an excellent index.

THE PRINCIPLES AND PRACTICE OF PSYCHIATRY.

*By Alexander Cannon and Edmund D. T. Hayes. William Heinemann (Medical Books), London.* 25 shillings net. $7\frac{1}{4}$ x $9\frac{1}{2}$; xvii + 437; 1933.

The authors of this book have had wide

experience in psychiatric work in English institutions. The purpose has been "to collect and to state in concise form the most recent knowledge in the realm of Psychiatry . . . . in a manner most convenient for teaching purposes, and for the preparing for the diplomas in Psychological Medicine of the various examining bodies." In the sixteen chapters hardly any phase of the subject of psychiatry has been neglected. This means that many topics are necessarily briefly treated. Nevertheless the book will serve exceedingly well those wishing to review the subject for examination. As a reference book physicians and lawyers will find it especially useful. The work contains many helpful diagrams and an appendix in which is collected much information which could not be conveniently included in the text. Unfortunately no bibliography has been included. Considering the extensive amount of material in the book the index seems hardly adequate.

THE PSYCHOLOGY OF ANIMALS *in Relation to Human Psychology*.

*By F. Alverdes. Harcourt, Brace and Co., New York.* \$2.25. $5\frac{1}{2}$ x $8\frac{1}{2}$; viii + 156; 1932.

Professor Alverdes contributes an interesting comparison of animal and human psychology to the field of popular scientific writing. The approach is essentially philosophical—that human beings are compelled to set up "fictions" in order to comprehend themselves and the world about them, that absolute knowledge is unattainable and only that knowledge can be attained which adapts itself to our sense organs and mental organization. The fiction is set up that, behind the whole life process, there is a psyche-like agent. Our consciousness would then be that region in which this agent is able to grasp itself. Since this is common to both man and animals mutual understanding is possible within limits. The limits depend on the fact that animal behavior is based on instinct whereas man is obliged to acquire by experience practically everything. As a rational contribution to a rather hackneyed but popular point of debate this book will interest the philosophically minded general reader.

WHAT WE PUT IN PRISON *and in Preventive and Rescue Homes*.

*By G. W. Pailthorpe. Williams and Norgate, London.* 5 shillings net. $4\frac{7}{8}$ x $7\frac{1}{4}$; 159; 1932.

This little book can scarcely be disregarded by those who have the difficult problem of dealing with the asocial individual. The author bases her work on a study of the psychology of 200 female criminals and inmates in prisons and preventive and rescue homes in England. She stresses the following points in any attempt to deal with such cases:—criminals and asocial persons should be looked upon as individuals who are suffering from psychological illness or defect; the unconscious motive of the delinquent should be carefully considered; no real progress in psychological cases can be made unless the offender's coöperation is enlisted; and, finally, a better understanding of the neurotic motive which too often builds and drives the machinery of reform. A large part of the book is taken up with case histories. The final chapters deal with conclusions and suggestions. A brief bibliography is given, also an index.

INSECT BEHAVIOUR.

*By Evelyn Cheesman. Philip Allan and Co., London.* 4s. 6d. net. $4\frac{3}{4}$ x $7\frac{1}{4}$; 189; 1932.

The argument which is developed in this book, somewhat obscurely and without careful discrimination between established fact and theory, is that insects live and move in a subconscious world of their own, comparable to the postulated human subconscious, and that there is progressive development of insects' mental faculties from the simplest to the highest. The latter, as judged by the author from their behavior, have reached the border between the subconscious and the conscious. Awareness of failure in attaining a goal, with change of behavior to more successful tactics, is taken to imply a state of consciousness and is regarded as inexplicable on the instinct theory alone. Examples are cited to illustrate the occurrence of this phenomenon. There is an index but no bibliography.

BEHAVIOR MECHANISMS IN MONKEYS.
*By Heinrich Klüver. University of Chicago Press, Chicago.* $4.00. 6 x 9; xvii + 387; 1933.

This monograph presents the results of an intensive analytical study of the behavior mechanisms of monkeys. The viewpoint is primarily biological and foundations are laid for investigations of the neurophysiology of behavior. The author has devised valuable new techniques for use in experimentation. The results contribute to an exact knowledge of the perceptual world of an animal. The nature of the animal's perceptual organization is found to be surprisingly like that of man. There is a bibliography of 309 titles, and an index.

ABILITY IN SOCIAL AND RACIAL CLASSES. *Some Physiological Correlates.*
*By Roland C. Davis. The Century Co., New York.* $1.75. 6 x 8⅞; xiv + 114; 1932.

The influence of biological variations on intelligence among social and racial classes is the problem discussed in this book. In order to avoid dispute over the nature of intelligence the author selected groups of people whose intellectual levels have been fairly well established by the standard intelligence tests. The groups investigated were white and negro college students, white and negro feebleminded, city white children, negro children, and mountain children. All groups were located in the South. Three biological phenomena were examined biometrically: (1) speed of nervous conduction as measured by the Achilles tendon reflex; (2) tapping rate; and (3) "electrical resistance of the body to a small current passing from the back of one wrist to the back of the other." This latter function, it is believed, corresponds rather closely to metabolic activity.

The author states in his summary that the study "may be said to have shown that certain biological variations are advantageous for the possession of good intelligence." A not altogether unforeseen conclusion.

CONDITIONING FINGER RETRACTION TO VISUAL STIMULI NEAR THE ABSOLUTE THRESHOLD. *Comparative Psychology Monographs, Vol. 9, No. 3, Serial No. 43.*
*By Sidney M. Newhall and Robert R. Sears. The Johns Hopkins Press, Baltimore.* 75 cents. 6⅝ x 10; 25; 1932 (paper).

A report of an experiment designed to determine absolute sensory thresholds simultaneously by two techniques, the conditioned response and the psychophysical judgment with the method of constant stimuli. Several definite results came out of the experiment, to wit: very low intensities of retinal illumination can be conditioned; also intensities which vary around a subject's absolute sensory threshold are conditionable; and "an established conditioned response can be as sensitive an indicator of sensory stimulation as a practical psychophysical judgment."

## DE OMNIBUS REBUS ET QUIBUSDEM ALIIS

THE HILARIOUS UNIVERSE. *Being Angela's Guide to Einstein—and That Crush.*
*By Richard Dark. Basil Blackwell, Oxford.* 4s. 6d. net. 4¾ x 7½; 130; 1932.

This impudent treatise by the author of *Shakespeare—and That Crush* is dedicated "without permission but with the deepest respect" to the Astronomer Royal! Its alleged purpose is to supply a hypothetical child called Angela with the information necessary to pass the examination to get her G.G. astronomer's badge—G.G. meaning Girl Guide, the British equivalent to a Girl Scout. Dark writes the text and Derrick does the pictures. It is a ribald book they have made. It treats the history of ideas about cosmogony in an extremely disrespectful manner. Even Dr. Einstein, that wise and good man, is handled with ill-becoming levity. Students should not be permitted to read this book. Happily our sound and constructive professors will not care to read it. We try to be and believe that we are broad-minded and tolerant in our editorial work, but after all humor has its place, and that place is not to deride the very foundations of

science. If the words of Einstein, Eddington, Millikan, and Jeans—even their slightest words—are not to be held sacred and above profanation, then indeed is the underpinning of the Holy Temple of Science crumbling.

The Universe of Science.

*By H. Levy. The Century Co., New York.* $2.00. $5 \times 7\frac{1}{2}$; xiii + 224; 1933.

This book is a piquant instance of a mathematician reproving mathematics. The author, who occupies the chair of Mathematics at the Imperial College of Science, protests vigorously against certain fashionable tendencies in present day science. Such writers as Jeans, Eddington, Millikan and Smuts, he concludes, have "approached their problems against a background of outworn Idealist Philosophy none the less significant in its colouring because it has been unobtrusively though tacitly present." The cause of this tendencious idealism he finds in the dominance of the mathematician in experimental science, so that "Mathematical Physics, to many interpreters, has taken on the appearance almost of a separate science where facts about the world are *proved* rather than discovered by observation and experiment."

Levy's own viewpoint is naturalistic and inductive. The universe is not a tidy assembly of discrete entities but "an enormous interrelated dynamic muddle with intermittent patches of order and sanity." Man himself is not a detached intellect viewing the universe from an absolute point of vantage but an integral part of his environment, physical and social. In order to reach some measure of understanding we break up experience into isolated systems and deal with these separately. This is a necessary step but none the less dangerous. For we are always liable to forget that we have ourselves isolated these systems and to find something mysterious in the "emergence" of a molecule of water from the combination of atoms of oxygen and hydrogen.

This is a stimulating and clearly written treatment of the philosophy of science, which both the scientist and the layman will profit by reading. There is an index but no bibliography.